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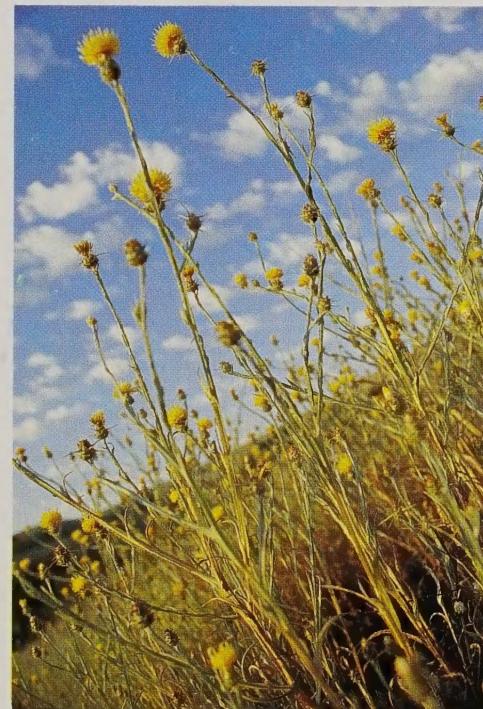
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Forest Health Technology Enterprise Team

Technology
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*Biological
Control*

Biology and Biological Control Agents of Yellow Starthistle



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Morgantown, WV



United States
Department of Agriculture



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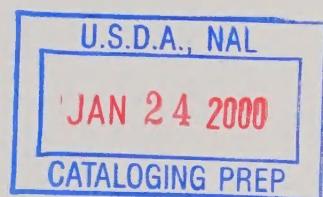
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Cover photo: Yellow Starthistle, *Centaurea solstitialis* L. infestation.

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Biology and Biological Control Agents of Yellow Starthistle



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How to Use This Manual

1. **Purpose.** This manual is intended as a reference guide for field workers and land managers to establish biological control agents in yellow starthistle fields. The guidelines outlined in this manual are based on research and experience in the field. Use the guidelines in conjunction with experience and common sense to optimize the success of the biological program.
2. **Format.** The manual begins with background information on biological control, program yellow starthistle and its biological control agents, followed by **HOW-TO** sections with techniques for implementing a biological control program. Throughout the manual, footers indicate the title or name of a section.
3. **Introduction.** This section contains information on yellow starthistle, its biological control and integrated vegetation management. It is not the intent of this manual to furnish detailed information on the complexities of rangeland management or biological control. The information contained in this publication is designed to assist field activities. For more detailed information, refer to the resources listed in the bibliography.
4. **Yellow starthistle.** Use this section to understand basic yellow starthistle biology and to identify the nine primary growth stages of yellow starthistle. Work with agents is scheduled by yellow starthistle growth stages so it is critical to be able to distinguish between them.
5. **Yellow starthistle weevil and fly biology sections.** Use these sections to understand and identify yellow starthistle agents. Because the three yellow starthistle weevil agents are similar, the first section contains biology information about weevils in general, followed by a table comparing the adult weevils and another table comparing the weevils' life cycles by growth stage of yellow starthistle. The subsequent sections contain information specific to each weevil, including identification, biology and life cycle, weed destruction potential, dispersal rate, common mistakes and comments (i.e., ways in which they are unique). This pattern is repeated for the two fly agents.

On the cover ...



Photo courtesy of Cindy Roche

6. **The How-to section.** Use this section for information about how to implement a biological control program for yellow starthistle. It encompasses techniques for all the agents. Information specific to each agent is listed separately in this section.
 - Each section begins with a summary of its contents.
 - Each section has a field supply list for planning field activities at the end of each section.

The following information is included:

- ✓ Work schedules for field activities
- ✓ Larva and pupa identification table
- ✓ Troubleshooting guide to establishment failures
- ✓ Establishing a biological control program for yellow starthistle
- ✓ Selecting and preparing a release or nursery site
- ✓ Monitoring agents and vegetation at the site
- ✓ Collecting, releasing, transporting, shipping, rearing, feeding and handling agents
- ✓ Establishing photo points

7. **Bibliography.** Use the selections to augment knowledge of biological control, yellow starthistle and its agents.
8. **Glossary.** Use the glossary to understand scientific terms.
9. **Appendix.** Use this section to supplement the information in the body of the manual.

Introduction

Yellow starthistle (*Centaurea solstitialis* L.) is a serious rangeland weed problem in northern Idaho. It is native to Eurasia and probably first came to North America in contaminated alfalfa or other crop seeds. Yellow starthistle seeds were found in adobe brick in California beginning in the early 1800's. There are several early records of yellow starthistle from university plant collections in California from the mid and late 1800's. First reports of yellow starthistle in the Pacific Northwest include an alfalfa field near Walla Walla, Washington, around the turn of the century. Yellow starthistle presently infests nearly 10 million acres in California, 500,000 in Idaho and 150,000 acres each in Oregon and Washington. Yellow starthistle continues to expand in Idaho at the rate of about 6% per year.

Yellow starthistle forms dense areas of blue-green color in the early spring which turn to yellow during the mid-summer (Figs. 1 and 2). These dense stands can reduce forage production and interfere with grazing. It competes with rangeland grasses, monopolizing nutrients, moisture and light.

Yellow starthistle primarily infests arid to semiarid rangeland and abandoned cropland, but also grows in grain fields, orchards, roadsides and recreational lands. Ingestion by horses can cause a chronic and potentially fatal neurological disorder known as "chewing disease". The spiny heads of the plant are a deterrent to grazing by livestock and an annoyance to hikers.



Fig. 1. Early spring yellow starthistle infestation shown in area of blue-green color.

Control Methods. Long-term, cost-effective, environmentally sound strategies for management of yellow starthistle may include a combination of cultivation, hand labor (where practical), herbicides, mowing during early flowering stage, grazing management, biological control and other practices that enhance competition by desirable vegetation. Well-adapted, perennial grasses can limit yellow starthistle invasion when they are well established. If the site does not have good perennial grass cover to take up resources released by yellow starthistle control, the habitat will likely become reinfested with yellow starthistle or other noxious weeds.

Biological Control. Much of the land invaded by noxious weeds is of low economic value, inaccessible or environmentally sensitive, which makes chemical or cultural control impractical. To maintain a weed species below economically damaging levels requires an aggressive vegetation management program that will include biological control.



Fig. 2. Flowering yellow starthistle near Peck, Idaho.

Yellow starthistle is not a problem in its native land for a variety of reasons, one of which is the presence of natural enemies to reduce population development. Classical biological control is the introduction and establishment of carefully selected natural enemies to exert stress on a noxious weed which ultimately causes plant death or reduces the competitive ability of the weeds to a point where desirable plant species can out-compete them. Few weed species have ever been satisfactorily controlled with purposeful introductions of biological control agents alone. The integration of various management techniques is the answer to reducing the dominance of yellow starthistle in susceptible habitats.

Survival of a large or small number of transferred natural enemies does not mean that immediate weed control will occur. The newly established natural enemies will serve as colonizers, and several years will be required for their descendants to reach population levels that can effectively stress the weeds. Currently, the USDA has approved three weevils: (1) *Bangasternus orientalis*, (2) *Eustenopus villosus*, and (3) *Larinus curvus*, and two flies: *Chaetorellia australis* and *Urophora sirunaseva* for release on yellow starthistle. These are seed consumers and all of them are currently established in Idaho, the Pacific Northwest, and California.

Integrated Management. Any one of the agents by itself will not eradicate or even control yellow starthistle. Merely reducing seed production will not kill the plants although the feeding activity may weaken the plants. A combination of biological control agents is needed to exert more stress on the yellow starthistle plants. The goal of biological control is not eradication but to reduce the competitive ability of the weed so that yellow starthistle-infested sites can be recolonized by desirable plant species. Biological control should, therefore, be part of a larger vegetation management effort that includes a combination of management techniques.

The advantages of using biological control are as follows:

- ✓ Once an agent is introduced, it reproduces without additional cost to the land manager.
- ✓ Agents move to host plants within the climatic range of their host.
- ✓ Approved biocontrol agents are selective and do not damage other vegetation, therefore, properly tested agents are not a source of environmental contamination.
- ✓ Reduction in use of herbicides.
- ✓ Low cost per acre.
- ✓ Weed pests are unable to develop significant resistance to biological control agent.

The disadvantages of using biological control are as follows:

- ✓ Establishing and increasing biological control agent populations to levels that impact the plant population is a 10- to 20-year commitment.
- ✓ Predators or parasites sometimes threaten agent populations.
- ✓ Environmental conditions that are excessive such as insufficient moisture or heat, may prevent establishment of an agent at a specific site.
- ✓ Biological control alone cannot eradicate a yellow starthistle population; multiple control methods are necessary.

Some biocontrol agents have limited availability at this time.

Yellow Starthistle

SCIENTIFIC NAME *Centaurea solstitialis* L.

COMMON NAMES Yellow starthistle, St. Barnaby's thistle, Golden thistle, Cotton-tip thistle.

FAMILY Sunflower family (Asteraceae or Compositae). Includes dandelion, sunflower, safflower, artichoke and wild chicory.

DESCRIPTION A winter-hardy annual that reproduces entirely by seed production (Figs. 3 and 4).

- ✓ **Height** The normal rangeland size is 1-3 feet, 4-6 feet tall in shady wet areas, or as short as 2 inches in dry areas.
- ✓ **Leaves** Basal leaves form a rosette. Each leaf is divided into lobes with the end lobe larger and rounder than the side lobes, the stalk shorter than the leaf blade. Stem leaves attach directly to the stem by a wing that runs down the side of the stem; they are up to 4 inches long and ¼-inch wide, linear or tapered at both ends with the broadest part below the middle.
- ✓ **Heads** Each flower bud appears as a small, egg-shaped swelling up to ¾-inch long and enclosed by shingle-like layers of bud scales called bracts. A sharp, yellow-green spine appears at the tip of each bract and develops with the bud to become ¼ to 2-inches long after the flowers fully open. Buds are solitary at the ends of the branches. The base of the head is pubescent (Fig. 5).
- ✓ **Stems** The stems are upright, stiff, winged and branched. Small plants usually have an unbranched stem and one flower head; large plants have a stem with many branches and can have over 100 flower heads.



Fig. 3. Yellow starthistle plant.



Fig. 4. Yellow starthistle seeds, plant, and leaves.

✓ **Flowers** Bright yellow flower heads, about 5/8 inch in diameter (Fig. 5).

✓ **Seeds** Tan with white and brown mottling, about 1/8-inch long. Seeds are of two types: (1) those with a white plume and (2) those without a white plume. Plumed seeds have a ring of fine, white, 1/8-inch long bristles, are slightly extruded and easily knocked from the seed head by wind or other disturbances. Plumed seeds are not readily airborne, but rely of wind, water, and animals for long-distance dispersal. Most plumed seeds drop on the soil near the mother plant. Plumed seeds may germinate as soon as moisture and temperature conditions are favorable. Seeds produced in flowers around the periphery of each head are smaller, dark-colored, plumeless and remain in the head until dissemination in the winter, providing a second method of seed dispersal (Fig. 6).



Fig. 5. BU-4 bud (L) and flowering (R) yellow starthistle heads.

Yellow starthistle can produce up to 150,000 seeds per plant. About 95% of the seeds produced are viable. From 20% to 40% of the seeds may remain alive after one year, and about 10% can lie dormant in the soil for up to 10 years.



Fig. 6. Yellow starthistle seeds.

GROWTH AND DEVELOPMENT OF YELLOW STARTHISTLE. The synchrony of agent activity with specific plant growth stages is an important factor in the success of biological control. Nine primary growth stages of yellow starthistle (see Fig. 7a-l) are described below for use in its management.

1. **Seedling stage** (Fig. 7-a) Germination begins in the fall and continues through spring and is followed by the emergence of two oblong cotyledons or seed leaves. The plants then produce 5 or more basal leaves and 2 to 4 deeply lobed leaves.
2. **Rosette stage** (Fig. 7-b) In the spring, 7 or 8 lobed leaves emerge to form a rosette as the plant grows in height and diameter ending with 20 or more leaves in the rosette.

3. **Bolting stage** (Fig. 7-c) The plant begins to bolt in late spring, sending up a rigid, winged flower stalk with a blue-green, cottony pubescence and tipped with a firm flower bud. The flower stalk can be simple in small plants, but is branched in larger plants. During this spring growth period, dense infestations of yellow starthistle that inhabit southern exposures of steep canyons may be identified from a distance by their characteristic blue-green color.
4. **Floral bud stage** From late spring to mid-summer, a developing branched inflorescence yields solitary buds at the ends of branches. Learning to recognize the four floral bud stages is important for biological control planning.
 - **BU-1** (Fig. 7-d) Small buds with yellow-green spines begin to be visible at the top.
 - **BU-2** (Fig. 7-e) Spines protrude more than $\frac{1}{2}$ of the bud length.
 - **BU-3** (Fig. 7-f) Spines are equal to or greater than 45° angle from stem.
 - **BU-4** (Fig. 7-g) Spines are straw-colored and equal to or greater than 90° angle from stem.
5. **Flowering stage** (Fig. 7-h) Bright yellow flowers appear in the summer.
6. **Seed formation stage** (Fig. 7-i) There is a progressive loss of color in mid-summer, but the bud still retains some green.
7. **Mature stage** (Fig. 7-j) By late summer, the leaves wither and dry, the bright yellow flowers fade, the plants take on a straw-colored appearance. Light-colored seeds are mature.
8. **Seed dissemination stage** (Fig. 7-k) From late summer to early fall, the flower head dries to a tan color, the bracts dry and release the plumed seeds which are dispersed either by wind, water or by clinging to clothing, fur or feathers.
9. **Senescence stage** (Fig. 7-l) The final stage begins in the fall and continues through the following spring when the plants continue to dry and lose their leaves, becoming silver-grey skeletons with white, cottony buttons by December or January. The flower heads have lost most of their spines and the plumeless seeds by this time. Eventually the plant disintegrates.



Figure 7-a. Seedling



Figure 7-b. Rosette



Figure 7-c. Bolting



Figure 7-d. BU-1



Figure 7-e. BU-2



Figure 7-f. BU-3



Figure 7-g. BU-4



Figure 7-h. Flowering



Figure 7-i. Seed formation



Figure 7-j. Mature



Figure 7-k. Dissemination



Figure 7-l. Senescence

Fig. 7a-l. Growth stages of the yellow starthistle.

Yellow Starthistle Weevil Biology

ANATOMY Weevil adults have a segmented body of three parts (head, thorax and abdomen), one pair of antennae and three pairs of legs. They are hard-bodied, with tough, thick exoskeletons. Weevils possess two pairs of wings; the front pair is thickened to form a hard covering called elytra. When the insects are not flying, the elytra are held over the back to form a protective covering. The membranous hind wings are used for flight and fold under the elytra when not in use.

Weevils generally have long, well-developed snouts with chewing mandibles at the tip. The antennae are attached to the snout about halfway along its length. They use the snout to feed internally on plant tissues and notch out egg-laying sites.

LIFE CYCLE Weevils develop and grow through a series of molts: egg, three or four larval instars, pupa and adult. Weevils are univoltine, meaning they complete one generation per year. The adult overwinters in protected areas on the ground and become active again in the spring. Thus, the first weevils are from the overwintering generation and the new weevils that emerge in the summer will overwinter until the next spring.

- **Adults** Most weevils overwinter as adults in the ground litter at yellow starthistle sites and emerge in the spring, however, some weevils will overwinter in the seed heads. Typically, males emerge 1 to 2 weeks earlier than females. This is important to remember for collection purposes because it is difficult to distinguish between male and female weevils. To ensure that both males and females are released, collect adult weevils after mating has been observed, usually 1 to 2 weeks after emergence (times differ for each weevil). During the first week after emergence, adult weevils are relatively inactive and spend most of their time on the ground. Sweeping is the best way to determine presence of adult weevils.
- **Eggs** Once mating has occurred and fertilized eggs are mature, the female lays (oviposits), the eggs. The eggs hatch within 3 to 21 days after oviposition depending on the species of weevil and environmental conditions. Weevil eggs are oval, yellowish, approximately 1.08 mm (0.04 in) long and 0.61 mm (0.02 in) wide and are deposited externally in the case of *B. orientalis* and *L. curtus*, or in cavities the female creates in the buds as in *E. villosus*. With experience, dissection of the seed head for eggs can be used to determine the presence of weevils.
- **Larvae** Larvae are different from the adults. Larvae have a different form, lack compound eyes, have reduced antennae, and lack external evidence of wing formation. Larvae specialize in eating whereas adults specialize in reproduction

and dispersal. Weevil larvae feed and develop, going through 3 to 4 molts or instars within the yellow starthistle bud, causing damage to the developing seeds. Larval development requires 16 to 20 days depending on the species of weevil and environmental conditions. Weevil larva is a white, legless, C-shaped grub with a brown head capsule. The first instar larva is approximately 1 to 2 mm (0.04 to 0.08 in) long; the last instar larva is approximately 5 to 7 mm (0.19 to 0.27 in) long. The larva is mobile and moves around within the seed head. A larva can only be seen by dissecting the seed head. It is impractical to distinguish between the weevil species' larva; however, it is possible to distinguish between fly and weevil larvae. Weevil larvae have a head capsule and fly larvae do not.

- **Pupae** Weevils pupate within a mature yellow starthistle seed head in a brown, dry-walled chamber composed of frass (excrement) and plant material. The pupa is approximately 4 to 6 mm (0.16 to 0.24 in) long and generally resembles the adult weevil. Appendages are present and free from the body, but the wings and elytra are shorter than on an adult and the pupa is immobile. They are white when newly formed, darkening with age. Pupation lasts from 4 to 13 days depending of the species of weevil. As with the larva, determining weevil presence by dissecting the seed head for pupae is time-consuming and impractical.
- **New generation adults** The majority of new generation adult weevils emerge in late summer and feed and mate until diapause. Diapause is a physiological condition characterized by low metabolism, little or no development, increased resistance to environmental extremes and reduced activity. Diapause is induced and maintained mostly by environmental factors such as day length, temperature and moisture. Once diapause is initiated, it continues until favorable environmental conditions signal the end of diapause and development and reproduction are resumed in the spring.

Adult weevils can be seen from early spring through late fall. More than one generation of a weevil species can be observed at a site at one time. More than one species can also be observed at one site. Table 1 compares the three yellow starthistle adult weevils for use in identification. Table 2 compares the three weevil life cycles at each growth stage of yellow starthistle for use in field activities.

Table 1. Comparison of Yellow Starthistle Adult Weevils

<i>Bangasternus orientalis</i>	<i>Eustenopus villosus</i>	<i>Larinus curtus</i>
		
Emerges 1 st (bolting)	Emerges 2 nd (BU-I)	Emerges 3 rd (BU-1)
Cylindrical body shape	Cylindrical-oblong body shape	Oblong body shape
4-6mm	4-6mm	5-6mm
Brown with yellow/white hairs	Brown with grey/white hairs	Brown
Mottled appearance	Striped	Yellow, spotted
Non-hairy looking	Hairy-looking	Pollen-covered
Short snout	Long, slender snout	Medium-sized snout
Release: BU-1 to BU-3 stages	Release: BU-3 to BU-4 stages	Release: flowering stage

Table 2. Comparison of Weevil Life Cycles and Monitoring Activities by Growth Stages of Yellow Starthistle

YST Stage	<i>Bangasternus orientalis</i>	<i>Eustenopus villosus</i>	<i>Larinus curtus</i>
Seedling	Adult overwinters in the ground litter. Not practical to find in the field.	Adult overwinters in the ground litter. Not practical to find in the field.	Adult overwinters in the ground litter. Not practical to find in the field.
Rosette			
Bolting	Adult emerges; find on plants. Monitor for overwintering generation emergence.		
BU-1	Adult oviposits eggs; find black egg cases. COLLECT MATING ADULTS.	Adult emerges, feeds on buds; find on plant. Monitor for overwintering generation emergence. Find feeding damage: wilted, bent or dead buds.	Adult emerges; find on plant. Monitor for overwintering generation emergence.
BU-2			Find adults on plants.
BU-3	Larva develops; find in seed head. Adults, egg cases seen on plants. COLLECT MATING ADULTS.		Find adults on plants.
BU-4	Larva develops; find in seed head. Find adults, egg cases on plants.	Adult oviposits eggs. COLLECT MATING ADULTS. Find feeding damage. Find oviposition scar. Find frass plug.	Find adults on plants.
Flowering		Larva develops; find in seed head. Find feeding damage. Find oviposition scar. Find missing flower section.	Adult oviposits eggs. COLLECT MATING ADULTS. Find adult buried snout down (posterior end up) in flower.
Seed formation			Larva develops; find in seed head. Find adults on plants. Chewing damage in flower.
Mature	Pupa pupates; find in seed head. Find adults, egg cases on plants.	Pupa pupates; find in seed head. Find adults on plants.	Pupa pupates; find in seed head. Find adults on plants.
Dissemination	Adult emerges; find on plants. Monitor for new generation emergence.	Adult emerges; find on plants. Monitor for new generation emergence.	Adult emerges; find on plants. Monitor for new generation emergence.
Senescence	Adult overwinters in ground litter. Not practical to find in the field.	Adult overwinters in ground litter. Not practical to find in the field.	Adult overwinters in ground litter. Not practical to find in the field.

Bangasternus orientalis

(Capiomont) (Coleoptera: Curculionidae)

COMMON NAME Seed weevil or yellow starthistle bud weevil.

BIOLOGY

Generations per year One.

Adult weevils are 4 to 6 mm long with a cylindrical-shaped body. Compared to *E. villosus*, it has a short snout. The weevil is dark red to brown with yellow to whitish hairs giving it a mottled appearance (Fig. 8).



Fig. 8. *B. orientalis* adults have a cylindrical-shaped body and the shortest snout of the three weevils. Yellow to whitish hairs give the weevil a mottled appearance.

Overwintering adults Adults emerge from overwintering sites during the yellow starthistle bolting stage. The adults are inactive during the cool morning hours, but become active and appear on plants during the warm hours of the day. Sweeping at any time will be successful if weevils are present. Although adult weevils feed on the leaf margins, damage to the yellow starthistle by adult feeding is minimal.



Fig. 9. *B. orientalis* egg.

Ovipositing adults Oviposition begins within one or two weeks of emergence during the BU-1 bud stage of yellow starthistle and continues for 4 to 8 weeks. Eggs are laid singly on terminal leaflets, the bases of young flower buds and on or near BU-1 and BU-2 buds. The female covers the egg with a dark green mucous material combined with fecal particles and plant hairs, which turns black and hard with exposure to the air. Although eggs are usually laid near the buds on the underside of leaflets, at high weevil populations much of the plant can be covered with eggs. More than one egg can be laid per bud, but usually only one larva develops per bud. Eggs are identified externally by the distinctive, teardrop-shaped, black protective cap and are a good way to determine weevil presence any time after the BU-1 bud stage (Fig. 9).



Fig. 10. *B. orientalis* pupal chamber.



Fig. 11. Exit hole of *B. orientalis*.

Larvae Eggs hatch within two weeks after oviposition and the larva enters the tissues of the host plant by tunneling superficially through the plant tissue, feeding along the way until a bud is reached. The larva then enters the flower at its base, develops and feeds on the receptacle tissue and developing seeds.

Pupae The weevils pupate in the flower heads in a thin-walled, brown pupation chamber. The pupal chamber can be observed in the seed head (Fig. 10).

New generation adults The new generation adult emerges by chewing an exit hole in the top of the infested BU-4 bud. Adults overwinter mostly in the ground litter to emerge again in the spring (although a small percentage overwinters in the seed head). The exit holes are pencil-shaped, and can be recognized with experience (Fig. 11).

Weed Destruction Potential

- Although adults feed on the leaf margins, the impact to the plant by adults is negligible.
- Internal larval feeding in the flower head reduces the number of developing seeds by 40% to 60% overall and 60% to 90% within an infested seed head.

Agent Dispersal Rate

- *B. orientalis* is a good flier and disperses well.

Comments

- *B. orientalis* is an easy agent to work with and may be the best agent to gain experience in collection, redistribution and development of nursery sites.
- *B. orientalis* is difficult to establish at sites where *E. villosus* is already established due to the predatory nature of *E. villosus*. When sharing a seed head with *E. villosus*, *B. orientalis* tends to have a high larval mortality.

Eustenopus villosus

(Boheman) (Coleoptera: Curculionidae)

COMMON NAME Yellow starthistle hairy weevil.

Adult *E. villosus* weevils are cylindrical to oblong, brown with longitudinal, grey to whitish stripes and have long hairs on the back. The weevils are 4 to 6 mm (0.16 to 0.24 in) long and have long, slender snouts (Fig. 12).

BIOLOGY

Generations per year One.

Overwintering adults Adult weevils emerge from the soil during the BU-1 bud stage of yellow starthistle. Typically, males emerge 1 to 2 weeks earlier than females. This is important to remember for collection purposes because it is difficult to distinguish between male and female weevils. To ensure the release of both males and females, collect adult weevils after mating and some oviposition has been observed. During the first week of their emergence, the adult weevils are relatively inactive and spend most of their time on the ground. Both male and female weevils feed on BU-1 through BU-4 buds (Fig. 13). This behavior is unique to *E. villosus* among the yellow starthistle weevils. This feeding damages the plant and is indicated by wilted heads, buds bent at an angle to the branch or dead buds (Figs. 13, 15 and 20). Adults usually begin mating on BU-3 and BU-4 buds.



Fig. 12. Adult *E. villosus* have longitudinal stripes and long hairs on their back. They have the longest snout of the three weevils.



Fig. 13. Adult *E. villosus* feeding on BU-1 bud.

Adults are active during the day, but less active in the morning. They can be found on all parts of the plants above ground, especially the buds. *E. villosus* is the slowest moving of the yellow starthistle weevils, but on a warm day they can easily escape nets and collecting trays.

Ovipositing adults Female weevils usually lay one egg per bud. She chews a hole above a spine at the base of a bract on an early BU-4 yellow starthistle bud, lays a single egg in the hole and then plugs the hole with frass. The presence of frass is an indication of the presence of *E. villosus* (Fig. 14). Sometimes the holes are made but not used for oviposition; the hole is covered with frass only when an egg is laid. Tan to brownish oviposition scars or scabs on the side of the bud (Figs. 14 and 20) is another indicator of the presence of *E. villosus*. Eggs are not visible externally; however, the eggs are visible with the naked eye or a 10X-hand lens in a dissected BU-4 bud. The egg hatches within 3 to 4 days.



Fig. 14. *E. villosus* oviposition hole.

Larvae Weevil larva develops and feeds on the receptacle of the bud during flowering and seed formation stages, causing damage to the developing seeds (Fig. 17). Larval development lasts 16 to 19 days. External indicators of the presence of *E. villosus* at this stage are oviposition scars (Figs. 14 and 20) and missing portions of the seed head (Fig. 19).



Fig. 15. Bent heads indicating *E. villosus* feeding damage.

Pupae Pupation occurs in a chamber within the seed head and lasts 8 to 13 days (Fig. 18).



Fig. 16. *E. villosus* egg.



Fig. 17. *E. villosus* larva.



Fig. 18 *E. villosus* pupa.



Fig. 19. Missing flower section indicates *E. villosus* damage.

Weed Destruction Potential

- *E. villosus* has excellent biocontrol potential because of the dual impacts of adult and larval feeding.
- External adult feeding may reduce the number of buds per plant by 75%.
- Internal larval feeding may reduce the number of developing seeds per head by 75% to 100%.

Agent Dispersal Rate

- *E. villosus* is a poor flier and needs to be released at more sites than the other weevils.

Comments

- *E. villosus* is one of the easiest weevils to work with because the adults are slower moving and present on more plant stages than the other weevils.
- *E. villosus* larvae are aggressive predators and eat whatever is in their feeding path, potentially displacing *B. orientalis* in the seed head and, ultimately the entire site. A more compatible combination of agents at a release site is *E. villosus* and *C. australis*.



Fig. 20. Oviposition scar and bent head indicate *E. villosus* damage.



Fig. 21. Darkened pappus hairs indicate *E. villosus* damage.

Larinus curtus

(Hochhut) (Coleoptera: Curculionidae)

COMMON NAME Yellow starthistle flower weevil.

Adult *L. curtus* weevils are 5 mm to 6 mm (a quarter of an inch long), medium-brown, oval-shaped. They have a medium-sized snout compared to *B. orientalis* and *E. villosus* and are often pollen-covered, giving them a yellow, spotted appearance (Fig. 22).

BIOLOGY

Generations per year One.

Overwintering adults Adult weevils appear from overwintering during the BU-1 bud stage. Sweeping is the best way to determine presence of adult weevils. Adults at this stage are relatively sluggish and are found on closed buds. During the hottest part of the day, they rest in shady places on the plants such as under the leaves.

Adults feed and mate more actively on open flowers and are easily found with its hind end sticking up in the yellow starthistle flower (Fig. 23).

Ovipositing adults The female weevils must feed on yellow starthistle flowers to stimulate development of the ovaries and egg production. Oviposition begins during the flowering stage of the yellow starthistle. The female prepares an opening among the florets and oviposits a few millimeters above the receptacle. One egg per flower head is the usual. Eggs are



Fig. 22. Adult *L. curtus* weevils have a medium-sized snout compared to the other two weevils. They are usually covered with pollen, giving them a yellow spotted appearance.



Fig. 23. Adult *L. curtus* are found with their hind ends sticking up in the flowers.

attached to a floret and covered with a dark, brown-black substance. Eggs hatch in about 4 days. The oviposition period is long, covering the entire flowering period.

Larvae The weevil larva develops during the seed formation stage and feeds within the capitula on the receptacle and developing seeds. *L. curtus* has four larval instars. Larval development lasts from 17 to 20 days. Experience and careful inspection of the seed head will yield evidence of adult weevil chewing in the center of the flower.

Pupae Pupation occurs in a chamber within the mature seed head and requires 4 to 5 days to complete.

New generation adults The new generation of adults begins to appear during the seed dissemination stage of yellow starthistle, enters diapause and overwinters outside the seed head in the ground litter (although a small percentage overwinter in the seed head).

Weed Destruction Potential

- Adults feed on flowers and pollen, but the impact is minimal.
- Internal larval feeding reduces the number of developing seeds in the bud by 96% to 100%.

Agent Dispersal Rate

- *L. curtus* adults are good fliers and have an excellent dispersal rate.

Comments

L. curtus is an important yellow starthistle biocontrol agent because it oviposits at a later stage of yellow starthistle development than the other agents (thereby destroying seeds that escape other yellow starthistle agents) and because the larva feeds directly on the developing seeds.

Yellow Starthistle Fly Biology

ANATOMY

Adult flies have an exoskeleton, a segmented body in three parts (head, thorax and abdomen), one pair of antennae, and three pairs of legs. Unlike weevils, adult flies have only one pair of wings on the middle segment of the thorax, and are excellent fliers. Also, unlike weevils, flies are soft-bodied and more delicate. Mouthparts are of the sponging type to take in liquid food.

The two fly agents for yellow starthistle, *Chaetorellia australis* and *Urophora sirunaseva*, belong to the family Tephritidae, the fruit flies (see Table 3). Tephritids have spotted or banded wings and are small to medium sized.

LIFE CYCLE

Flies, like weevils, undergo complete metamorphosis: egg, three larval instars, pupa and adult. Yellow starthistle flies usually have two generations per year, although *C. australis* can have three generations per year if the site has a long growing season. The overwintered generation emerges from a seed head during the bolting stage and then feeds, mates, and oviposits. The summer generation adults emerge from a seed head during the BU-3 stage and feed, mate and oviposit. The larva overwinters in the seed head of the yellow starthistle plant to continue the cycle the following spring.

Adults

Adults are found on yellow starthistle plants and other flowers where they obtain nectar. There are two or more generations of adults per year. Sweeping or sighting can determine the presence of flies. Because flies are delicate, collecting adult flies with sweep or aerial nets can cause some mortality. To reduce mortality, it works best to make two sweeps, aspirate the flies into the breathable container for transportation, make two more sweeps, aspirate, etc., until finished. Determining presence by sighting adults is possible; however, not seeing the agent does not necessarily indicate its absence.

Eggs

Female flies lay up to 250 eggs that hatch within 8 to 12 days. Fly eggs are generally elongated, white or pale yellow and are deposited on a closed yellow starthistle bud usually under or between bracts. With experience, dissection of the seed head for eggs can be used to determine presence of flies; however, this detection technique is not feasible for the novice.

Larvae

The fly larva is white, turning yellowish with maturity, legless, conical-shaped without a head capsule, slightly thicker at one end with two black spots on the thicker end, and measure from 0.5 mm to over 4 mm in length. Larva feeds and develops through 3 instars inside the yellow starthistle seed head. When a larva has sufficiently built up its food reserves, it transforms into a pupa. Summer generation larva diapauses and overwinters in the seed head and pupates in the spring.

The fly larva lacks the head capsule present on weevil larva. A microscope and experience are required for the task of distinguishing between *C. australis* and *U. sirunaseva* larvae. Note, however, that *U. sirunaseva* larva forms a distinctive hard, barrel-shaped gall while *C. australis* larvae do not have galls.

Pupae

The immobile pupa is concealed within a barrel-shaped puparia, which is light yellow with darker end segments. Pupa averages 3.5 x 1.7 mm. The pupal stage occurs inside the yellow starthistle seed head and lasts about 2 to 3 weeks. Determining fly presence by dissection of the seed head for pupa is possible, but impractical on a large-scale basis.

Table 3 compares the two yellow starthistle adult flies for use in identification. Table 4 compares the two fly life cycles at each growth stage of yellow starthistle for use in field activities.

Table 3. Comparison of Yellow Starthistle Flies

<i>Chaetorella australis</i>	<i>Urophora sirunaseva</i>
Straw-colored body	Black body
Black spots on body	Yellow spots on thorax
Straw-colored wing bands	Black wing bands
2-3 generations per year	2 generations per year
Oviposits inner side lateral bracts	Oviposits on top of buds
Release: BU-3 bud stage	Release: BU-2 thru BU-3 bud stage

Table 4. Comparison of Fly Agent Life Cycles and Monitoring Activities by Growth Stage of Yellow Starthistle

YST Stage	<i>Chaetorellia australis</i>	<i>Urophora sirunaseva</i>
Seedling	Larva diapauses; find in old seed heads. Dissect seed heads for larva. Collect seed heads with old bracts or florets attached.	Larva diapauses within gall. Presence of larva: intact bracts, woody gall, dark spot in pappus hairs.
Rosette	Pupa in seed head. Dissect seed head for pupa. Collect seed heads with old bracts or florets attached.	Dissect seed heads for galls. Collect seed heads with old bracts or florets attached.
Bolting	Adult emerges; find on plants. Monitor for overwintering generation emergence.	Adult emerges; find on plants. Emergence hole in gall. Monitor for overwintering generation emergence and emergence hole.
BU-1	Adult oviposits eggs. Monitor site for adult fly activity.	
BU-2	Egg hatches 2-4 days. New larva tunnels, develops, feeds on seeds. Pupa pupates. Find adults on plants. Monitor site for adult fly activity. Dissect seed head for larva or pupa.	Adult oviposits eggs. Egg hatches 2-4 days. New larva tunnel, develop, feed on seeds. Pupates. Find adults on plants. Monitor site for adult fly activity. Dissect seed head for larva or pupa. COLLECT ADULTS.
BU-3	New generation adult emerges, oviposits. Find adults on plants. COLLECT ADULTS.	New generation adult emerges, oviposits. Egg hatches 2-4 days. Larva develops, feeds on seeds. Find adults on plants. COLLECT ADULTS.
BU-4	Egg hatches 2-4 days. Larva tunnels, develops, feeds on seeds. Find adults on plants. Monitor site for adult fly activity. Dissect seed head for larva or pupa.	Larva develops, feeds on seeds. Presence of larva: intact bracts, woody gall, dark spot in pappus hairs. Monitor site for adult fly activity.
Flowering	Larva develops, feeds on seeds. Dissect seed heads for larva.	Larva develops, feeds on seeds. Dissect seed heads for woody galls.
Seed formation	Larva diapause;s find in seed heads.	
Mature		
Dissemination	Dissect seed heads for larva. Infested seed heads with bracts intact.	Larva diapauses. Dissect seed heads for woody galls.
Senescence		

Chaetorellia australis

(Hering) (Diptera: Tephritidae)

COMMON NAME Peacock fly.

Adult *C. australis* flies have straw-colored bodies with eleven black spots and straw-colored bands on their wings (Fig. 24). They are approximately 3 to 6 mm (0.12 to 0.24 in) long. Females are typically longer than the males and have an ovipositor.



BIOLOGY

Generations per year Two or three generations depending on the length of the growing season.

Overwintering generation *Chaetorellia australis* overwinters in yellow starthistle seed heads as a mature larva and pupates at the same time as the yellow starthistle rosette stage.

The overwintered generation adults emerge during the yellow starthistle bolting stage and feed on available nectar. At sites where bachelor's button (*C. cyanus*) is present, the first generation will oviposit and develop within the bachelor's button seed head.

Summer generation adults Summer generation adults generally emerge during the BU-3 stage of yellow starthistle, although adults are out as early as the bolting stage feeding on nectar of various plants. Female flies oviposit during the BU-3 or BU-4 bud stage. Eggs are laid singly at the lateral walls of the closed capitules beneath an involucral bract of a flower head. The eggs are white, spindle-shaped and have a long characteristic filament thickened at the distal end, which can extend beyond the margins of the bract. Eggs hatch within 2 to 4 days. The development from egg to adult lasts about 4 weeks.

Summer generation larvae The fly larvae tunnel through the involucre into the interior



Fig. 25. *C. australis* larva.

Summer generation larvae The fly larvae tunnel through the involucre into the interior of the flower head, where they feed on the ovaries and developing seeds during the BU-4 through flowering stage (Fig. 25). The larvae feed, develop, diapause and overwinter in the yellow starthistle seed heads.

Weed Destruction Potential

- Internal larval feeding reduces the number of developing seeds in the bud by 80% to 100%.

Agent Dispersal Rate

- Excellent dispersal rate.
- Caution: May already be present at some sites.

Comments

- Bachelor's button was thought to be necessary for optimum establishment of *C. australis*. Although the fly is established at most sites without bachelor's button, it is possible that preference for bachelor's button at emergence could be a limiting factor at some sites.
- *C. succinea* is easily confused with *C. australis*. With experience, however, it is easily distinguished from *C. australis* by an extra dot on the thorax. *C. succinea* has 5 dots on each side of the thorax, *C. australis* has 4 dots on each side of the thorax (see Fig. 26). *C. succinea* is currently established in various sites in Idaho, but it is currently not approved as a yellow starthistle agent since there is the potential to adapt to attacking safflower crops.

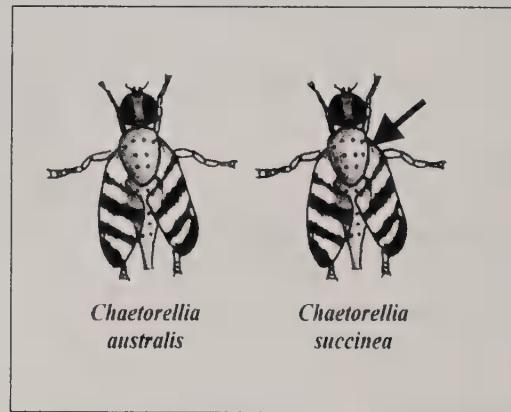


Fig. 26. *C. succinea* is distinguished from *C. australis* by the extra dot on the thorax.

Urophora sirunaseva

(Hering) (Diptera: Tephritidae)

COMMON NAME Yellow starthistle seed head gall fly.

BIOLOGY

Generations per year Two.

Adult flies are black with a yellow triangle on the back of the thorax. Wings are marked with dark crossbands. The adults are approximately 3 to 6 mm (0.12 to 0.24 in) long. The female is typically longer than the male and has an obvious ovipositor (Fig. 27).

Overwintering generation *U. sirunaseva* overwinters in galls in the yellow starthistle seed head as a mature larva and pupates inside the gall in the spring; pupation lasts 4 to 5 weeks.

First generation adult flies emerge from galls in the spring, about 2-4 weeks after *C. australis*, and begin mating within 3 to 4 days. Females then begin oviposition. Eggs are laid on the top of BU-2 or BU-3 buds where the points of the smaller bracts emerge. The eggs are white and spindle-shaped, with one end narrower and longer than the other end. The average sized egg is about 1 x 0.15 mm. The eggs hatch within 9 or 10 days.

Summer generation larvae After hatching, the larvae eat through the florets in the head, and can be found on and between the florets. When the larvae reach the receptacle, gall formation begins. The tissues begin to grow and change consistency, forming a protruding cell with a small opening at the far end (Figs. 28 and 29). There is one larva per gall and 2 to 4 galls per seed head. When the larvae finish eating, the walls become lignified, protecting the mature larvae. The gall walls of the summer generation larvae are delicate compared with the thicker gall walls formed by the overwintering generation.



Fig. 27. Adult female (L) and male (R) *U. sirunaseva* are black with a yellow triangle on the thorax. Wings are marked with dark bands. Females have an ovipositor.



Fig. 28. Dark spot indicating *U. sirunaseva* gall.

Larva and pupa can be found by dissecting the seed head. Presence of a woody gall within a seed head is an indication of presence of the agent (Fig. 29). A dark spot in the pappus hairs also indicates an *U. sirunaseva* gall (Fig. 28).

Summer generation pupae Pupation lasts about 2 to 3 weeks in both generations.

Summer generation adults The summer generation adults emerge in late June and early July and begin oviposition within 3 days. An emergence hole in the gall can be detected (Fig. 30).

Overwintering generation larvae Larval development in this generation differs from the summer generation in that the larvae form much thicker galls. The larvae feed and develop for about three weeks. Once the larvae reach maturity, they enter diapause and overwinter in the gall formed in the yellow starthistle seed head for approximately seven months.



Fig. 29. *U. sirunaseva* woody gall.



Fig. 30. Emergence hole of *U. sirunaseva*.

Weed Destruction Potential

- Internal larval feeding reduces the number of developing seeds in the head by approximately 50%.
- Heads infested with gall flies produce fewer seeds due to the limited amount of receptacle for seed production and the galls create a resource sink on the entire plant so that the overall number of heads produced is reduced.

Agent Dispersal Rate

- Excellent disperser traveling several miles annually.

Comments

- The presence of high populations of *U. sirunaseva* at a site does not appear to interfere with the survival of other seed head infesting agents.
- *U. sirunaseva* may be confused with *U. affinis* in locations where diffuse and spotted knapweed grow with yellow starthistle.

How to Establish a Biological Control Program

Follow these steps to establish a biological control program for yellow starthistle:

- 1. Read background information**
 - 2. Obtain current information**
 - 3. Schedule field activities**
 - 4. Select the release site**
 - 5. Verify source of agents**
 - 6. Monitor agents at the release site**
 - 7. Collect and release agents**
 - 8. Solve establishment problems**
-

1. *Read background information* - Read the information contained in this manual and become familiar with biological control, yellow starthistle and its biological control agents. You must be able to identify yellow starthistle and its growth stages, each agent and their life stages, the agents and what they are doing and when. Timing of the collection and release is important; therefore, pay close attention to the WORK SCHEDULE in Tables 5 and 6.
2. *Obtain current information* - Contact the local weed supervisor, county extension agent, USDA Animal and Plant Health Inspection Service (APHIS), a Plant Science or an Entomology Department at a State University for current information regarding yellow starthistle, biological control, and sources of biological control supplies or agents.
3. *Schedule field activities* - Follow the timetables Tables 5 and 6 to schedule field activities.
4. *Select the release site* - See the section on "How to Select a Release or Nursery Site."
5. *Verify source of agents* - Contact the agent supplier to verify availability of agent(s). If purchasing or obtaining biological control agents from a supplier, order the agents the year before the proposed release date. If collecting, ensure that the source is established and available for redistribution.
6. *Monitor agents at the release site* - See the section on "How to Monitor Sites for Bioagents."

7. *Collect and release agents* - The activities of biological control agents are synchronized with yellow starthistle growth stages; monitoring yellow starthistle growth stages at the site will more accurately determine collection and release dates. Familiarity with the growth stages of yellow starthistle is crucial to coordinating work with the agents.
 - Times to collect and release agents will vary by agent. Use the timetables in Tables 5 and 6 as a guideline for scheduling field activities before and after release.

Table 5. Timetable for Pre-Release Year Activities

Proposed Pre-Release Year Activities		
YST Growth Stage	Activity	Relevant Section (How to ...)
BU-1 to flowering stage	Monitor potential collection and release site(s) to determine presence or absence of agents. Select potential collection and release sites.	<i>Monitor Sites</i> <i>Select a Release or Nursery Site</i>
Seed dissemination stage	Order agents (optional).	<i>Establish a Biological Control Program</i>

- Yellow starthistle growth stages vary by year and by geographic area. For the purposes of this manual, field activities are scheduled by yellow starthistle growth stages, not by date. Because many different stages of yellow starthistle occur in the field at the same time, stages refer to the dominant stage of yellow starthistle. Use the dates listed in "Schedule of Dates of Yellow Starthistle Growth Stages in Idaho and Washington" (Table 7) as a general guideline for scheduling work activities. The dates were derived from previous years of experience in Idaho and Washington at about 1,000-foot elevation. Sites at lower elevations will be about 3 or 4 weeks earlier than the dates listed.

 8. *Solve establishment problems* – If there is a problem with establishing the release site with bio-agents use the "Troubleshooting Guide to Establishment Failures" (Table 11).

Biology and Biological Control Agents of Yellow Starthistle

Biology and Biological Control Agents of Yellow Starthistle

Table 6. Timetable for Release and Post Release Year Activities

Proposed Release and Post Release Year Work Schedule		
YST Growth Stage	Activity	Relevant Section (How To ...)
Seedling	Collect/distribute/rear yellow starthistle seed heads for <i>C. australis</i> and <i>U. sirunaseva</i> .	<i>Collect Seed Heads for Releasing Flies</i> <i>Release Fly Infested Seed Heads</i>
Bolting stage	Photograph release sites. Monitor release sites for emergence of overwintering generation of <i>B. orientalis</i> and <i>C. australis</i> .	<i>Establish a Photo Point</i> <i>Monitor Sites</i> <i>Monitor Sites for Bioagents</i>
Bolting stage to BU-3	Monitor vegetation.	<i>Monitor Vegetation</i> <i>Conduct Qualitative Monitoring</i> <i>Conduct Semi-Qualitative Monitoring</i> <i>Conduct Quantitative Monitoring</i>
BU-1	Determine collection/release dates. Monitor release sites for emergence of overwintering generation of <i>U. sirunaseva</i> .	<i>Collect Adult Biocontrol Agents</i> <i>Monitor Sites for Bioagents</i>
BU-1 to BU-3	Determine collection/release dates. Collect and release <i>B. orientalis</i> Monitor release sites for emergence of overwintering generation of <i>E. villosus</i> .	<i>Monitor Sites</i> <i>Monitor Sites for Bioagents</i> <i>Collect Adult Biocontrol Agents</i> <i>Monitor Sites</i> <i>Monitor Sites for Bioagents</i>
BU-2 to BU-3	Determine collection/release dates. Collect and release <i>U. sirunaseva</i> . Dissect seed head for larva or pupa of <i>C. australis</i> and <i>U. sirunaseva</i> .	<i>Monitor Sites</i> <i>Collect Adult Biocontrol Agents</i> <i>Monitor Sites for Bioagents</i>
BU-3	Determine collection/release dates. Collect and release <i>C. australis</i> . Monitor release sites for emergence of new generation of <i>C. australis</i> and <i>U. sirunaseva</i> .	<i>Monitor Sites</i> <i>Collect Adult Biocontrol Agents</i> <i>Monitor Sites for Bioagents</i>
BU-3 to BU-4	Collect/release <i>E. villosus</i> . Monitor release sites for emergence of overwintering generation of <i>L. curtus</i> . Photograph release site (optional).	<i>Collect Adult Biocontrol Agents</i> <i>Monitor Sites</i> <i>Establish a Photo Point</i>
Flowering	Collect and release <i>L. curtus</i> .	<i>Collect Adult Biocontrol Agents</i>
Seed dissemination	Monitor release sites for emergence of new generation of <i>E. villosus</i> , <i>B. orientalis</i> , <i>L. curtus</i> . Visual count of emergence or exit holes made by <i>E. villosus</i> exiting the pupal chamber. Determine level of infestation of 100 seed heads for <i>C. australis</i> and <i>U. sirunaseva</i> .	<i>Monitor Sites</i> <i>Yellow Starthistle Biology</i> <i>Monitor Sites for Bioagents</i>

Biology and Biological Control Agents of Yellow Starthistle

**Table 7. Schedule of Dates of Yellow Starthistle Growth Stages
in Idaho and Washington**

YST Growth Stage	Approximate date in Idaho and Washington at 1000' elevation
Seedling	October to April
Rosette	Mid-April
Bolting	Mid-May to mid-June
BU-1	Mid-May to mid-June
BU-2	Early June to mid-June
BU-3	Mid-June to early July
BU-4	Early July to late July
Flowering	Mid-July to early August
Seed formation	Late July to late August
Mature	Early to late August
Seed Dissemination	Mid-August to early September
Senescence	Early Sept to spring

Biology and Biological Control Agents of Yellow Starthistle

How to Select a Release or Nursery Site

To select a release or nursery site, follow these five steps :

- 1. Gather supplies**
 - 2. Visit prospective sites**
 - 3. Obtain permission from the landowner**
 - 4. Determine the type of site**
 - 5. Choose an appropriate site**
-

1. **Gather supplies** – Prepare the following supplies to select the site:
 - Insect net
 - Permission form
2. **Visit prospective sites** – Visit the potential sites to collect information about the site. Use the following criteria to evaluate the potential of prospective sites as release or nursery sites:
 - **Presence of agent.** Check to ensure that agents are not already present at the collection site. If agents are present, choose another site.
 - **Size of site.** Select an area infested with at least 1-acre of yellow starthistle (approximately 200' x 200' or the equivalent).
 - **Density of infestation.** Moderately dense infestation of yellow starthistle (i.e. more than two yellow starthistle plants/m²).
 - **Traffic.** For nursery site, select a site free from farm equipment and vehicular traffic. For release sites give preference to sites with little or no traffic.
 - **Rodents.** Avoid areas with a major infestation of rodents. However, this is difficult in most places and sometimes cannot be avoided.
 - **Grazing.** For a nursery site, select an area free from grazing.
 - **Mowing.** Avoid areas where mowing occurs.
 - **Pesticides.** Be careful to select a site away from agricultural fields or a right-of-way to avoid pesticide drift.
 - **Habitat.** Choose sites with mixed habitats nearby (e.g., areas with full sun and partial shade) that are infested with yellow starthistle.

Biology and Biological Control Agents of Yellow Starthistle

- **Location.** Closest available site to the redistribution site for optimal establishment. If the site is to be used as a nursery site, initially release each species one-quarter of a mile apart.
3. *Obtain permission from the landowner* – There are three things to obtain from a landowner:
- **Permission.** Ask written permission from the landowner or land manager to establish a biological control site.
 - **Accessibility.** Make certain the landowner understands that the site must be accessible for monitoring and collecting for at least 6 years (3 years for establishment and buildup and at least 3 years for collections).
 - **Permanent location marker.** Property owners must allow the use of permanent markers, such as a brightly painted wood, metal or fiberglass stake, to mark the release site. Be certain to let the landowner know the location of the stake.
4. *Determine the type of site* - Determine whether the site is a nursery (field insectary) site or a release site. The difference between the two types of sites is focused on how the site will be used. The extent of management for each site will depend on program objectives and available resources.
- **Nursery site.** The focus at a nursery (or field insectary) site is on establishing a biological control agent nursery. After the initial release, the site is left alone for 3-5 years to allow the agent populations to increase significantly. Sites may be monitored yearly to determine agent establishment; however, due to potential damage to agents, monitoring the vegetation is discouraged at nursery sites. After 3 years the agent population may be high enough to collect and redistribute the agents at other yellow starthistle sites. An ideal nursery site is an area where minimal disturbance is anticipated.
 - **Release site.** Release sites are either *monitored* or *unmonitored*.
- A *monitored* release site is focused on monitoring the impact of the agent on the weed at a specific site. The information gathered is used to determine the effectiveness of the program and the impact of the biocontrol agent on weed populations. Frequent monitoring activities will take place at monitored release sites.
- An *unmonitored* release site consists solely of an initial release and little, if any, follow-up. The purpose of a non-monitored release site is to get the agent into the yellow starthistle areas.
5. *Choose an appropriate site* – Choose a site that best fits the criteria listed above.

How to Prepare a Release Site

To prepare a release site, do the following:

- 1. Gather supplies**
 - 2. Establish a permanent location marker**
 - 3. Set up a photo point**
 - 4. Draw a map to the release site**
 - 5. Record the location information**
 - 6. Establish baseline vegetation monitoring data**
-

1. *Gather supplies* – Get the following supplies to take to the site:
 - Agent release form
 - Pencil
 - Photo point supplies (optional)
 - Vegetation monitoring supplies
 - Wood, metal or fiberglass fence post
 - Marking paint for post
 - Map of area or GPS unit
2. *Establish a permanent location marker.* This involves locating a spot and placing a marker on that spot.
 - **Locate spot.** After the site is selected, locate a spot that has a dense infestation of yellow starthistle (> 2 plants/m²) and is also suitable for use as a photo point (see “How to Establish a Photo Point”).
 - **Marker.** Place a tall, brightly colored wooden, fiberglass or metal stake at the approximate center of the release site for future identification. Attempt to locate a spot that best serves all purposes. Do not use anything that is temporary or potentially difficult to see such as cairns or rebar. It must be tall enough to be seen at future visits. Metal stakes can sometimes be unsuitable for the site due to the potential for damaging equipment.
3. *Set up a photo point.* A photo point will record the change in the site over time. For information about photo points and setting up a photo point on the release site, see “How to Establish a Photo Point”.

4. *Draw a map.* Draw a map and write down the directions to the release site for future use. Do not use descriptions like 'adjacent to Farmer Brown's field'. Use legal descriptions or Global Positioning Satellite (GPS) coordinates along with permanent roads, creeks, rivers, mile markers, etc. Remember that the next person to visit the site may be unfamiliar with your landmarks and descriptions.
5. *Record location information.* Record information about the site on the University of Idaho Agent Release Form (Appendix A). Send a copy of the final form to the agency indicated on the form.
6. *Establish baseline vegetation monitoring data.* If the vegetation on the site is to be monitored, choose a method listed on "How to Monitor Vegetation." Establish baseline data the season before releasing the agent(s). It is important to use the same method at the same plots every year.

How to Monitor Sites

Address the following questions when conducting a biological control program:

- 1. Are the biological control agents present at the site?**
 - 2. Are the agents found in high enough numbers to be collected and redistributed?**
 - 3. Are the agents causing visible damage to the target weed?**
 - 4. Are changes occurring within the plant community?**
-

Monitoring the release sites will answer the questions above and accomplish the following:

- ◆ Determine the relative success of biological control efforts for target weed populations.
- ◆ Establish that biological control agents are impacting the target plant.
- ◆ Document changes in the plant community.
- ◆ Demonstrate that biological control efforts may be partially responsible for the changes observed.
- ◆ Determine collection and release dates.

The type and intensity of monitoring used will vary with the question being addressed and how precise an answer is required. The monitoring methods described are not the only methods available; however, they are commonly used and have been found to be useful. The two types of monitoring are as follows:

1. **Agent monitoring.** Use agent monitoring to determine the presence, absence and/or establishment of an agent.
 - Use "How to Monitor Sites for Bioagents" as a guide for monitoring the agents at a site.
2. **Vegetation monitoring -** Since the release and management of biological control agents against yellow starthistle has a management purpose related to the amount or extent of this noxious weed, vegetation monitoring is an important part of the biological control strategy. Use vegetation monitoring to determine whether the release of biological control agents is effective in meeting management objectives.
 - Use "How to Monitor Vegetation" as a guide for monitoring the vegetation at a site.

How to Monitor Sites for Bioagents

Monitoring for agents is important for many reasons. Since many biological control agents are capable of long range dispersal, it is important to determine whether it is already present before releasing an agent at a given site. It is also important to determine whether a released agent was able to reproduce and remain on a site. Some agents can be detected the year following a release. Some agents take 2 to 3 years to build a population to detectable levels. Use agent monitoring to determine the presence, absence and/or establishment of an agent.

Monitoring for agents determines collection and release dates. To monitor sites for agents, do the following:

- 1. Gather supplies**
 - 2. Determine when and how often to monitor for agents**
 - 3. Monitor for agents using a visual survey method**
 - 4. Monitor for agents using the sweep net survey method**
 - 5. Monitor for agents using the larval or pupal stage method**
 - 6. Determine the level of agent presence**
 - 7. Troubleshoot establishment problems**
 - 8. Fill out the monitoring form**
-

1. **Gather supplies** – Get the following supplies to take to the field to monitor for agents:
 - Monitoring form
 - Pencil
 - 15" canvas or aerial sweep net
2. **When and how often to monitor** – The time to monitor for each agent is variable from season to season, site to site and with experience in the field. Consider the following to decide when to monitor a site:
 - **Ideal sampling times.** If resources permit, sample at the following periods: (1) two weeks before estimated peak emergence, (2) at peak emergence, and (3) two weeks after peak emergence. Peak emergence of the agent will be the most successful time to determine the presence of an agent even though adult agents can be seen from the bolting stage throughout the summer. The exact dates or number of times to monitor per season will depend on each biocontrol program and the available resources.

A GOOD TIME TO WORK WITH ALL THE AGENTS IS WHEN THE YELLOW STARTHISTLE IS AT 5-10% BLOOM. For *B. orientalis*, *C. australis* and *U. sirunaseva*, one to two weeks earlier than 5% bloom of yellow starthistle is the optimal time. The optimal time for *E. villosus* and *L. curtus* is at 5-10% bloom.

- **Sampling conditions.** Good conditions for sampling adult agents are a sunny, warm ($> 70^{\circ}\text{F}$) day; calm or with just a slight breeze. Plan to arrive at the site after 10 a.m. when the vegetation is dry and the dew is gone as heavy moisture interferes with observations.
- **Suggested times to monitor adult agents.** Monitor the yellow starthistle growth stages at the site using Tables 8 and 9 as guides for monitoring adult weevil and fly agents.

The three frequently used agent monitoring methods (visual survey, sweep net and larval or pupal stage) are described below. Use a method that best fits each agent and the appropriate growth stage of yellow starthistle.

3. *Visual survey method* – Use a visual survey method to determine the presence or absence of an agent. When determining presence or absence, keep in mind the following: (1) results will vary greatly between observers and the observer's expertise; (2) for population estimates, the less subjective sweep net and seed head sampling methods are recommended, and (3) the visual survey method is recommended for use by experienced monitors.
 - **Locate the permanent location marker.** If monitoring at a site with no previous release, mark a temporary point with a stake or clipboard.
 - **Visual count.** Make a visual count of all adult agents seen during three separate 5-minute transects along the collection site from the permanent location marker. Use Tables 8 and 9 to determine how to spot an agent and the best time to look for the agent. When making a visual count keep in mind the following observations:
 - ✓ Individual weevils or mating pairs can be located on stems, terminal leaflets, and young flower buds of yellow starthistle plants.
 - ✓ Some agents are inactive and difficult to see during the cool morning hours, however, sweeping at any time during the day will be successful if the agents are present.
 - ✓ Flies can be observed on and above the plants.
4. *Sweep net survey method* – Use the sweep net survey method to determine presence/absence or establishment of an agent. This is the preferred method for *L. curtus* when yellow starthistle is not at the flowering stage. During the flowering

stage, the agent is easily found with its hind end sticking up in the yellow starthistle flower. At other stages it is necessary to thoroughly examine each plant to determine the presence of *L. curtus*.

- **Look.** Spend a few minutes looking over the site for evidence of adult agents, especially in and on the buds or flowers.
- **Locate the permanent location marker.** Use a temporary stake, clipboard or data sheet to mark a point in the middle of the weed population for sweeping monitoring areas.
- **Sweep.** Using a 15" canvas sweep net, gently sweep the top half of the yellow starthistle buds in a 180° arc. Sweep the yellow starthistle plants at five checkpoints along four lines at least 25 feet apart or in four cardinal directions (N, S, E, W) from the location marker. Begin the lines as close to the location marker as possible.
- **Sweep.** Make four sweeps in front of the body (back and forth twice).
- **Look.** Examine the net for the specific agent.
- **Count.** Count and record the number of agents present.
- **Dump.** Empty the net to release the agents.
- **Move.** Move 5 feet (2-3 paces) and repeat.
- **Repeat.** Continue for five total points per line.

5. *Larval or pupal stage monitoring method* – During the larval and pupal stages, presence or absence of an agent can also be determined by the collection of seed heads. This method can be used for weevils, but is most commonly used with flies as the window for collecting fly infested seed heads is longer than with the weevils.

- **Collect.** Collect seed heads following the guidelines in "How to Collect Seed Heads for Releasing Flies."
- **Sampling by seed head collection.** There are two methods for sampling agents by seed head collection:
 - (1) Rear agents in the laboratory in a rearing cage. Count and record the number of flies.
 - (2) Dissect seed heads and identify, count, and record the number and species of larvae and pupae found.

See Table 10 for help in identifying larvae and pupae.

6. *Level of agent presence* – Use the following guidelines to determine whether the agent is present, absent or established:

- **Presence.** Agents are PRESENT if any agents are found. If present, let the site continue to develop.
- **Absence.** Agents are ABSENT if no agents are found. Failure to see the agent does not necessarily indicate agent absence. If absent, decide whether to revisit the site to confirm absence, redistribute more agents or abandon the site. Make the decision to abandon the site AFTER resolving why the site failed to establish.
- **Establishment.** Agents are WELL ESTABLISHED if 10-15 agents are swept per each set of six sweeps. If the site is established, the site can be used as a collection site for distribution to other sites (see "How to Collect Seed Heads for Releasing Flies"). If agents are not established, see "Troubleshooting Establishment Failures" (Table 11).

As a general rule, some researchers suggest not returning to a release site for 2 to 3 years to determine success or failure. A release is not considered unsuccessful if no agents were detected the year after release. Revisit the site another 1 to 2 years before determining the success or failure of a release.

7. *Troubleshoot establishment problems* – Use "Troubleshooting Establishment Failures" (Table 11)as a guideline for problems in the field. The troubleshooting guide is compiled from past experiences in establishing agents
8. *Monitoring form* - Fill out the following data on a suitable monitoring form in the Appendix and return to the appropriate monitoring agency:
 - **Date.** Enter the month, day, year of sampling.
 - **Time.** Record the approximate local time of sampling.
 - **Weather conditions.** Give the best estimates (or measure, if possible) of air temperature and wind velocity and direction.

Table 8. Suggested Timing for Monitoring Adult Weevil Agents

YST Stage	Monitor for	Activity
<i>Bangasternus orientalis</i>		
Bolting – seed dissemination	Presence, absence or establishment	Look or sweep for adults (Fig. 7-d)
Bolting	Overwintering generation adult emergence	Look or sweep for adults
BU-1 thru BU-3 PEAK EMERGENCE	Determine collection and release date	Look for mating adults Look for black, tear-shaped egg cases (Fig. 9)
Seed dissemination	New generation emergence	Look or sweep for adults
<i>Eustenopus villosus</i>		
BU-1 thru seed dissemination	Presence, absence or establishment	Look or sweep for adults (Fig. 12) Look for adult feeding damage: wilted heads, dead buds, buds bent at an angle (Fig. 16; Fig. 20)
BU-1 BU-2	Overwintering generation adult emergence	Look or sweep for adults Look for adult feeding damage
BU-3 thru BU-4 PEAK EMERGENCE	Determine collection and release date	Collect when weevils are mating
BU-4 thru seed dissemination	Presence, absence or establishment	Look or sweep for adults Look for adult feeding damage (Fig. 15; Fig. 20) Look for oviposition scars, (Fig. 14; Fig. 20) frass plugs (Fig. 14; Fig. 20) missing flower sections (Fig. 19)
Seed dissemination	New generation emergence	Make a visual count of emergence holes. Record # of exit holes seen during 15-minute time period. Record distance from permanent location marker
Seed dissemination thru senescence	Presence, absence or establishment	Look for oviposition damage as darkened area among pappus hairs (Fig. 21)
<i>Larinus curtus</i>		
BU-1 thru seed dissemination	Presence, absence or establishment	Look or sweep for adults (Fig. 22)
BU-1 thru BU-4	Overwintering generation adult emergence	Look or sweep for adults
Flowering PEAK EMERGENCE	Determine collection and release date	Look for mating adults Adult chewing damage in center of flower Look for the dark hind end of the weevil sticking up from the yellow flower (Fig. 23)
Seed dissemination	New generation emergence	Look or sweep for adults

Table 9. Suggested Timing for Monitoring Adult Fly Agents

YST Stage	Monitor for	Activity
<i>Chaetorellia australis</i>		
Bolting – BU-4	Presence, absence or establishment	Look or sweep for adults (Fig. 24)
Bolting	Overwintering generation adult emergence	Look or sweep for adults
BU-3 PEAK EMERGENCE	Determine collection and release date	Collect when flies are active
Seed dissemination	New generation emergence	Look or sweep for adults
<i>Urophora sirunaseva</i>		
Bolting – flowering	Presence, absence or establishment	Look or sweep for adults (Fig. 27)
Bolting	Overwintering generation adult emergence	Look or sweep for adults
BU-2 thru BU-3 PEAK EMERGENCE	Determine collection and release date	Look for mating adults Collect when present and active
BU-3	New generation emergence	Look or sweep for adults Look for emergence hole (Fig. 30) Look for evidence of gall (Figs. 28 and 29)

Table 10. Larva and Pupa Identification

Agent	YST Stage	Description
<i>Bangasternus orientalis</i>	BU-2 thru seed maturation	Larva C-shaped, cream-colored with light brown head capsule. Confines feeding activity in seed head. Forms pupal chamber from frass (Fig. 10). Larval damage to seeds 40% to 60%.
<i>Chaetorellia australis</i>	BU-4 thru rosette	Larva flattened and dirty lemon yellow color (Fig. 25). Larva found in the bottom of the receptacle and embedded in pappus hairs. Larva moved from seed to seed eating contents of individual seeds and form a round hole. Pappus hairs severed and matted together with secretions. Seed is found partially chewed. Multiple larva per seed head. Pupa pupate in bottom of receptacle. Larval damage to seeds 80% to 100%.
<i>Eustenopus villosus</i>	Flowering thru seed maturation	Larva C-shaped, cream-colored with light brown head capsule (Fig. 17). Pupal chamber like little pots (Fig. 18). Leaves a mass of chewed-up debris in the bottom of the receptacle. Forms chamber from debris and glue-like substance. Larvae predatory. Larval damage to seeds 75% to 100%.
<i>Larinus curtus</i>	Seed formation thru maturation	Larva C-shaped, cream-colored with light brown head capsule. Leaves a mass of chewed-up debris in the bottom of the receptacle. Larval damage to seeds 96% to 100%.
<i>Urophora sirunaseva</i>	BU-2 thru rosette	Larva squat and creamy, off-white color with dark brown spiracular plate. Forms a woody, one-chambered gall. Larval damage to seeds 50%.

Table 11. Troubleshooting Guide to Establishment Failures

Problem	Possible Cause	Solution
Releasing unhealthy agents at site due to transportation stress	Physical damage to agent	Protect transport container from physical damage by taping down potential harmful objects such as blue ice containers. Use crushproof breathable container such as an ice cream carton.
	Drowning	Feed agents water by using a wrung-out paper towel or sponge. No excess water around agents.
	Excess heat and asphyxiation	Keep agents cool. Keep agents well ventilated out of the sun.
	Transport conditions	Keep agents cool and well ventilated. Keep agents from being crushed.
	Duration of transport	Release agents as soon as possible after collection. Work with shipping carrier regarding timetables to ensure a minimum amount of time in transportation.
	Starvation	Provide agents with fresh yellow starthistle stems or sugar solution.
Source of agent	Parasitism Disease	Use reliably disease free sources whenever possible.
Agent past reproductive age	Quality and number of eggs low	Collect at times of the highest possible agent fecundity.
Sex ratio: collect too many males	Males emerge earlier than females	Observe mating behavior in agent before collection to ensure the release of females at the site.
Agent synchrony: collect too early or too late	Females not in oviposition stage	Collect and release during correct life stage (oviposition stage) of each agent.
Host synchrony: weed at incorrect stage for female to oviposit.	Agent dies or leaves to find correct stage	Release agent at proper yellow starthistle growth stage for each agent.
Collection method: physical damage to agent.	Sweeping	Handpick agents, if possible. Use the dry bud placement method for delicate flies (pp. 76-82).
Site characteristics (abiotic factors)	Climate	Release at favorable site location.
	Elevation	Release at favorable site location.
	Soil	Release at favorable site location.
	Latitude	Release at favorable site location.
	Frequency of fire	Avoid releasing in sites with known fire patterns.
	Grazing	Avoid releasing in sites where livestock graze.
	Frequency of flooding	Avoid releasing in areas of flooding.
	Pesticide use	Avoid areas of known pesticide use.

Table 11. Troubleshooting Guide to Establishment Failures (continued)

Problem	Possible Cause	Solution
Site characteristics (biotic factors)	Native parasitoids EUVI larvae eat BAOR larvae	Avoid releasing in areas of known native parasitoids.
	Spider webs	Avoid releasing in areas of spider infestations.
	Natural enemies	Avoid releasing in areas of known natural enemies.
	Grasshoppers	Avoid releasing in areas of known high grasshopper populations.
	EUVI larvae eat BAOR larvae	Establish agents at separate sites.
Fly mortality	Delicate flies killed sweeping for beetles	Avoid establishing delicate flies at weevil multiple collection sites.
Social structure: behavior of agent	Can't find agent	Wrong time of day. Look between 1 and 6 p.m. Weather too rainy. Go to site during hot dry weather. Agent hiding. EUVI clings to plant. Look harder. Agent not there. Agent didn't like the site and moved elsewhere.
Procedural: pre-release	Site selection	Follow guidelines for proper site selection.
Procedural: release	Timing of release	Follow guidelines for correct release date.
	Quality of agent	Follow guidelines for caring for agent.
	Quantity of agent	Release a minimum of 100 agents per site.
	Density of plants	Site should have moderately dense infestation of YST.
	Density of agent	Release in areas with no agents present or of low density.
	Method of release	Cage flies. Release flies on ground and let them crawl up. Release flies on colder temperature so they can warm up slowly and crawl around site before flying.
Procedural: post release	Site management	Document site location properly.
	Failure to detect or identify agent	Train field workers adequately.
	Turnover in personnel	Use returning personnel as much as possible.

How to Evaluate Bioagent Feeding Damage

One method to determine whether the biological control agents are present or impacting the weed population is to collect yellow starthistle plants and evaluate agent feeding damage. Follow these directions:

- 1. Gather supplies**
 - 2. Determine conditions for collecting plants**
 - 3. Determine a sampling point**
 - 4. Collect plants for evaluating agent feeding damage**
 - 5. Evaluate feeding damage of agents**
-

1. *Gather supplies* – Prepare the following supplies to take into the field:
 - Data sheets
 - Pencil
 - Sharpie
 - GPS unit (optional)
 - Large (13 gallon) plastic bags
2. *Conditions for collection* - Ideal conditions for collecting yellow starthistle plants to evaluate agent feeding damage are:
 - **Warm day.** A sunny, warm ($> 70^{\circ}\text{F}$) day, calm or with just a slight breeze.
 - **Dry vegetation.** Plan to arrive at the site after 10 a.m. when the vegetation is dry to reduce the possibilities of mold on the collected plants from the morning dew.
3. *Sampling point* - The point will vary depending on the site.
 - **Marker.** At release sites, the sampling point is the exact agent release point or permanent location marker.
 - **Temporary marker.** At monitoring areas, the sampling point is a point in the middle of the weed population that can be marked with a temporary stake or object such as a clipboard or data sheet.
4. *Collect plants* – Collect yellow starthistle plants using the following steps:
 - **Select random plants.** Select six plants at random along four lines in four cardinal directions (N, S, E, W) from the permanent location marker. Select

plants by walking a predetermined number of paces from the permanent location marker, then collecting the nearest plant. The method must allow for random selection of yellow starthistle plants along the transect. The length of transect may vary with the type of site (release site versus monitoring site). Collect the entire above-ground portion of the plant. Pull the plant out by the roots and shake off the excess dirt.

- **Bag plants.** Place the plants in a bag as collected, using one bag per transect.
 - **Label bag.** Label the bag with a release code (if applicable), or site name, date and transect.
 - **Record data.** Using the GPS unit, record the latitude and longitude and elevation (if available) of the site on a data sheet.
5. Evaluate feeding damage. Evaluate the feeding damage to yellow starthistle by counting the flower buds. Flowering yellow starthistle plants will exhibit signs of presence of most of the biological control agents.
- **Count.** In the laboratory, count and record the total number of buds by stage (i.e., BU-1, BU-2, etc.), flowers and mature seed heads for each plant.
 - **Examine.** Examine each bud carefully for signs of infestation or damage using "Larva and Pupa Identification" (Table 10) and the Weevil and Fly Biology sections as references. Count and record how many buds were infested or damaged.

How to Monitor Vegetation

What is vegetation monitoring? Monitoring consists of multiple estimates of a variable(s) or attribute collected over time and analyzed to determine if a change occurred. In this case we are interested in changes in specific variables of yellow starthistle infestations as a result of the release of biocontrol agents. Vegetation monitoring at specific sites will depend on the stated management objectives, available resources and an initial assessment of the proposed treatment.

Monitoring is generally grouped into three intensity levels: qualitative, semi-quantitative and quantitative. Study the following material to determine the method that may be useful for a specific site. Follow-up with the specific section for each of the intensity levels.

- 1. Qualitative monitoring**
 - 2. Semi-quantitative monitoring**
 - 3. Quantitative monitoring**
 - 4. Developing a monitoring objective**
 - 5. Developing a monitoring checklist**
-

1. **Qualitative monitoring** - The collection of descriptive elements about the target population and/or management site.
 - It includes such general elements as presence or absence, density classes, estimates of age classes, distribution classes, infestation mapping and permanent photo points.
 - Qualitative monitoring is quick and therefore inexpensive, and provides some insight on the status or change of the yellow starthistle population.
 - Interpretations often are subjective, and the descriptive nature does not allow for quantitative analysis.
2. **Semi-quantitative monitoring** - The collection of population or site variables that are actually measured or counted, but not at a level where the precision of the monitoring can be determined.
 - The measured attribute is used as an index to track changes in a population.
 - Data collected over time can be compared qualitatively.
 - Semi-quantitative monitoring may be used to trigger more intensive monitoring.

- ✓ A single permanent quadrat or transect established at a release site where the number of flowering yellow starthistle is determined is an example of semi-quantitative monitoring.
3. *Quantitative monitoring* - The sampling of population/site variables that are measured or counted, and the precision and adequacy of the sample data can be calculated.
- Adequate samples from a defined population or site are collected and estimates of population parameters are calculated.
 - The population is sampled over time to determine population trends.
 - Quantitative monitoring is designed to be repeatable, allows for statistical analysis of trends and the calculation of sampling precision.
 - Quantitative monitoring takes more time to plan and implement, making it more expensive. It may also require expertise in quantitative methods and analysis.
4. *Monitoring objective* – Develop a monitoring outline before collecting data.
- The basis for the plan will be the management objective for the target weed and the monitoring objective.
 - The monitoring objective will determine if you are achieving the management objective as a result of releasing biological control agents.
 - The monitoring objectives need to identify the population that is being monitored, the variables or attributes that will be measured, and the frequency of the measurements.
- Select one of the following two types of monitoring objectives:
- ✓ Low intensity monitoring levels such as qualitative and semi-quantitative monitoring need stated objectives. Since low intensity monitoring is not designed for statistical analyses, it will have fewer elements in the objective statement. One example of a monitoring objective for low intensity monitoring is to determine at 3-year intervals the density class and distribution class of yellow starthistle at the Lenore Seed Orchard. Another example is to determine at the time of agent release and 3 year intervals thereafter, the density of yellow starthistle within the permanent quadrat at the Lenore Seed Orchard.
- ✓ Quantitative monitoring samples the yellow starthistle population to detect changes in some average value as a result of releasing biocontrol agents. The monitoring objective would specify additional elements related to the adequacy of the sampling approach. To provide essential information regarding the adequacy of the sampling it is necessary to include the following:
- ♦ Outline of the parameters to be sampled

- ◆ Population of interest
 - ◆ Level of detectable change in the parameters
 - ◆ Level of detectable change in the parameters
 - ◆ Confidence level of detecting a change
 - ◆ Acceptable probability of detecting changes when the attribute really did not change
5. *Monitoring checklist* - The following is a list of questions that need to be answered and documented prior to collecting monitoring data. Use this checklist as an outline for a monitoring plan. For the Monitoring Plan Checklist Form, see Appendix A.
- What is the management objective?
 - What is the monitoring objective?
 - What is going to be measured?
 - What is the appropriate level of monitoring intensity?
 - What is the population to be monitored?
 - Where will the monitoring take place?
 - When will the monitoring be conducted (month/year)?
 - How often will monitoring be conducted?
 - Who will conduct the monitoring?
 - How much will the monitoring cost (include summarizing and analyzing the data)?
 - How are the field data sheets to be designed?
 - What equipment and supplies are needed?
 - Is training necessary?

How to Conduct Qualitative Monitoring

To monitor a site using the qualitative monitoring method, follow the five steps listed below:

- 1. *Gather supplies***
 - 2. *Establish the monitoring site***
 - 3. *Estimate cover, density, and distribution classes***
 - 4. *Fill out site documentation form***
 - 5. *Establish a permanent photo point***
 - 6. *Set monitoring dates***
-
-

1. *Gather supplies* – Prepare the following supplies:
 - Monitoring form
 - Pencil
 - Photo point list
2. *Establish the monitoring site* - Establish the general area where the vegetation information will be collected. The area corresponds to where the biocontrol agents were released (the permanent location marker).
3. *Estimate cover, density and distribution class* - Estimate the canopy cover, density and distribution classes of yellow starthistle within the monitoring area. This is a visual estimate by class of the general release site. If the observers have not estimated general vegetation attributes, training may be necessary.
4. *Documentation Form* – Several examples of monitoring forms have been provided in the Appendix (A-G). Choose an appropriate form and fill it out.
5. *Photo Point* - Establish a permanent photo point in the monitoring area. Format the photo to capture the estimated cover and/or density classes of the yellow starthistle. Label the photo with the relevant estimated attributes.
6. *Set monitoring dates* - For comparison purposes, conduct vegetation monitoring at the same time of year at predetermined intervals established in the monitoring outline. Qualitative monitoring is not robust enough to track small yearly changes in weed population attributes; therefore, it may not be necessary to monitor vegetation each year.

How to Conduct Semi-Qualitative Monitoring

Permanent plots can be combined with qualitative monitoring to provide low cost estimates of change through the document of vegetation attributes at slightly different scales. To monitor a site using the semi-qualitative monitoring method, follow the eight steps listed below:

- 1. Gather supplies**
 - 2. Select the monitoring area**
 - 3. Set monitoring dates**
 - 4. Documentation form**
 - 5. Permanently monument the corners of the plot**
 - 6. Determine the bearing and distance to permanent location marker**
 - 7. Count the flowering or bolting yellow starthistle**
 - 8. Estimate percent cover**
 - 9. Analyze the measurements**
 - 10. Establish a photo point**
-

1. *Gather supplies* – Prepare the following supplies:
 - Data sheets
 - Pencil
 - Stakes
 - Hammer
 - Compass
 - Quadrat
 - Photo point supplies
2. *Select monitoring site* - Select an area at the release site that would represent the average vegetation condition of the site. The density or cover of yellow starthistle is representative of the site in general.
3. *Set monitoring dates* - For comparison purposes, conduct vegetation monitoring at the same time of year at predetermined intervals established in the monitoring outline.
4. *Documentation Form* – Several examples of monitoring forms have been provided in the Appendix (A-G). Choose an appropriate form and fill it out as measurements are taken.

5. *Monument plot corners* - Permanently monument the four corners of the plot if using a quadrat. Use wood or fiberglass stakes to establish the quadrat corners. Place the stakes to the outside of the plot corners or just inside the quadrat.
6. *Location of site* - Determine and record the bearing and distance from the site post to the permanent plot if a post or stake has been placed at the release site.
7. *Count yellow starthistle plants* - Count and record the flowering or bolting yellow starthistle within the quadrat. A 1-meter square quadrat provides a sufficient area to count plants.
8. *Estimate percent cover* - Estimate the percent cover of the target weed and dominant plants within the plot. Estimates could also be made by life forms if species is not known.
9. *Analyze measurements* - Compare the results between years. The changes that take place within the quadrat can be used as an index for changes that are occurring at the release site. The permanent plot approach is a single sample of the yellow starthistle attributes at the site. No averages or variation can be calculated from the single plot.
10. *Establish a photo point* - Establish a photo point of the permanent plot (see "How to Establish a Photo Point"). Format the photo to depict the entire permanent plot. Label the photo with the relevant estimated attributes.

How to Conduct Quantitative Monitoring

The intent of a macroplot design is to sample a portion of the macroplot area by randomly selecting a number of quadrats to be placed within the macroplot. See Appendix F for an example of a macroplot layout.

Quadrats are located within the macroplot by a set of randomly selected coordinates that correspond to the x and y axis. A specified pair of coordinates would be generated from a random numbers table. If a pair of coordinates repeat, the second set of coordinates would be dropped and another set would be selected. The number of samples would be based on the stated monitoring objectives, the variability of the target weed, and the size and shape of the quadrat.

The macroplot size depends on the size and shape of the quadrat. A total of 500 (1 X 2 ft.) non-overlapping quadrats could be placed within the macroplot.

To monitor a site using a macroplot design, do the following:

- 1. *Gather supplies***
 - 2. *Determine the quadrat shape and size***
 - 3. *Design the macroplot***
 - 4. *Select the coordinates at random***
 - 5. *Mark the quadrat on a macroplot layout***
 - 6. *Determine the compass bearing***
 - 7. *Position the quadrat***
 - 8. *Documentation form***
 - 9. *Count yellow starthistle stems***
 - 10. *Set monitoring dates***
-

1. Gather supplies – Prepare the following supplies to take into the field:

- Data sheets
- Pencil
- Random coordinates
- Meter tape
- Stakes
- Hammer

- Compass
 - Quadrat
 - Photo point supplies
2. *Determine the quadrat shape and size* - Rectangular quadrats are recommended because they are more precise in clumped vegetation. They should be a size and shape to capture the variation within the quadrats, rather than have a high variation between quadrats. The size of the quadrat will depend greatly on the density and distribution of the weeds within the proposed macroplot area, and how much change in the weed density to be detected is desired. A plot size of 2-3 square feet in a rectangular shape is a good initial quadrat for yellow starthistle of moderate density and even distribution.
 3. *Design the macroplot* - The sides of the macroplot need to be multiples of the sides of the quadrat. For example, a macroplot of 25 ft by 50 ft could be designed for a 1 x 2-ft quadrat. Place a total of 500 1x2 ft, of non-overlapping quadrats within the macroplot.
 4. *Select the coordinates at random* - Quadrats are located within the macroplot by a set of randomly selected coordinates that correspond to the x- and y-axis. Obtain coordinates by using a random numbers generator on a calculator or table. Select 2 numbers for each pair of coordinates that will correspond to the x- and y-axis along the macroplot. Select numbers until there is a set of coordinates for the number of sample units within the macroplot. The units of measure (i.e., feet, yard, pace) and the dimensions of the macroplot will determine the range of potential numbers that could be selected. Discard numbers that range farther than the size of the macroplot. Graph the coordinates on a diagram of the macroplot to provide a layout reference. Begin at the 0'0 coordinate, then work across the x-axis and up the y-axis to minimize disturbing the unmeasured quadrats.
 5. *Mark the selected quadrat* - Mark the selected quadrat on a macroplot layout (see Appendix E) to assist in efficiently locating the quadrats in the field.
 6. *Determine and record the compass bearing* - Place the y-axis tape parallel with the slope and upslope from the 0-ft mark. Monument the beginning and ending of the y-axis with a permanent marker. Place a second tape along the x-axis perpendicular to the y-axis. To lessen the risk of impacting a selected coordinate location before the quadrat is sampled, begin at the 0'0 coordinate corner and proceed across the x-axis and upslope along the y-axis.
 7. *Position the quadrat* - Position the quadrat so that the long side is parallel to the x-axis and adjacent to and above the tape (upslope side of the tape), with the lower-left hand corner corresponding to the set of coordinates.

8. *Documentation Form* – Several examples of monitoring forms have been provided in the Appendix (A-G). Choose an appropriate form and fill it out.
9. *Count the yellow starthistle stems* - Count the number of flowering stems of yellow starthistle with the quadrat positioned at the selected coordinates. Estimate the percent cover of plants species or general life forms. Record the attributes on a data form. Repeat this step for each selected location within the macroplot.
10. *Set monitoring dates* - Monitor at the same time of year at predetermined intervals as established in the monitoring outline. Select a new set of random coordinates each time the macroplot is sampled.

How to Establish a Photo Point

Photographs of the release site are a valuable assessment tool. Comparing pictures of the same site taken from the same location, at the same time of year, with the same horizon, and taken over a period of years provides visual evidence of vegetation change over time. Photographs are a qualitative method of monitoring and can be used in conjunction with more intensive monitoring approaches covered in "How to Monitor Vegetation."

- 1. *Gather supplies***
 - 2. *Take baseline photographs***
 - 3. *Locate the photo point***
 - 4. *Close-up pictures***
 - 5. *General view pictures***
-

1. *Gather supplies* – Prepare the following supplies to take into the field:

- Pencil
- 35-mm camera with a 28-mm wide-angle lens
- Color film; slides are preferred
- Photo identification label (close-up)
- One stake for camera point
- Orange paint for stake(s)
- Hammer

For close-up photos:

- Frame for close-up shots
- Stakes of $\frac{3}{4}$ or 1-inch angle iron >16" long (close-up)

2. *Take baseline photographs* - Take a set of photographs of the site the year before release. During the baseline vegetation monitoring is a convenient time to take the first set of pictures, however, the bolting and/or flowering stages are ideal stages to choose because of the color of the foliage.

- **Frequency.** Taking pictures of the site often is a good practice, but not essential. Once per year is suggested per release site (but take the pictures at the same time and same location every year).
 - **Color photos.** Photographs are to be in color using close-up and/or general view pictures. Color photos are preferred because they offer more information than black and white photos.
3. *Locate the photo point* - Locate and document the location of the photo point. The location of the photo point is determined at the time of establishing the release site. Establish and document a photo point marker for relocation purposes.
 4. *Close-up pictures* - Close-up pictures show the amount of ground covered by vegetation and litter.
 - **Camera.** Use a 35-mm camera with a 28-mm wide-angle lens.
 - **Camera point.** Locate the camera point on the north side of the photo, so photos can be taken at any time of the day without a shadow.
 - **Frame.** Generally a 3- x 3-foot square frame is recommended. Frames can be made of PVC pipe, steel rods, etc. Brightly painted angle iron stakes are driven into the corners to establish the plot permanently. Repaint the stakes when subsequent photos are taken.
 - **ID label.** Place an identification label on the ground next to the frame.
 5. *General view pictures* - General view pictures provide a broad view of the release site.
 - **Camera.** Use a 35-mm camera with a 28-mm wide-angle lens.
 - **Camera point.** Establish a camera point approximately 50 meters from the permanent location marker, keeping in mind to take photos that will best show changes in the yellow starthistle population over time. Mark the camera point with a brightly painted stake. Document the location of the camera point, height of camera, and time of day.
 - **Direction.** Take photographs of the site from the camera point toward the permanent location marker.
 - **Picture.** Include a photo identification label, a general view of the site, and some sky in the picture.
 - **Reference point.** It is important that the photo include a reference point in the foreground (fencepost, shrub, or person) and a distinct landmark on the skyline.
 - **Previous photos.** Previous year's photos are useful for duplicating the exact photo the following year.

How to Collect Adult Biocontrol Agents

In order to effectively collect the biological control agents, it is important to read and understand the background information pertaining to yellow starthistle and the yellow starthistle bioagent identification and life cycles before collecting. Follow these steps to collect adult biocontrol agents:

- 1. *Gather supplies***
 - 2. *Determine the collection site***
 - 3. *Determine the collection and release date(s)***
 - 4. *Obtain containers for collection***
 - 5. *Number of agents to collect***
 - 6. *Care of the agents***
 - 7. *Record data on the Agent Release Form***
 - 8. *Suggestions for optimal collections***
 - 9. *Common mistakes***
 - 10. *Sweep net collection method for weevils***
 - 11. *Sweep net collection method for flies***
 - 12. *Handpicking collection method***
 - 13. *Tap collection method***
 - 14. *Collection method specific to each agent***
-

1. *Gather supplies* – Gather the following supplies to take to the site:

- Agent release form
- Pen or pencil
- Marking pen
- Appropriate insect net
- Aspirator for flies
- Tape
- Small vials for voucher specimens
- 70% Ethyl alcohol for voucher specimens
- Breathable containers
- Newspaper/paper towel
- Cooler(s) and blue ice

- Funnel and bucket for hand-picking
 - Stick or badminton racquet and bucket for tap method
2. *Determine the collection site* – Check with the local county extension agent for available sites. Use the closest available site to the redistribution site whenever possible. To ensure that agents ARE ESTABLISHED at the collection site, follow the guidelines on “How to Monitor Sites.”
 3. *Determine the collection and release dates* - Monitor the collection site to determine a collection date (see “How to Monitor Sites for Bioagents). Use Table 6 as a guideline for scheduling collection and release dates for each agent. Consider the following when scheduling collection dates:

- **Mating weevils.** The weevils must be mating to be collected for release. It is difficult to differentiate between male and female weevils except during mating. If they are not mating and a collection is made too early, the collection may be mostly males and the new population will not establish itself at the release site. Observation of mating pairs ensures releasing both sexes at the release site.

Male weevils emerge one to two weeks earlier than female weevils. If the collection is made before the females emerge, the collection will lack the females to lay eggs at the release site and establishment of the site will fail.

If the weevil agents are collected before mating occurs, the agents could disperse before they have a chance to mate and adjust to the new environment, reducing the odds for establishment.

Male and female flies emerge at similar times; therefore, it is not a problem for collecting flies. The male and female flies are also easy to differentiate (see “Yellow Starthistle Biology”).

THE WEEVIL AGENTS MUST BE MATING TO BE COLLECTED FOR RELEASE

- **Bud stage.** Coordinate the mating of the agents at the collection site with the appropriate bud stage for the agent at the release site, (i.e., collect during flowering, release at a site where the yellow starthistle is flowering).
- **Warm day.** A sunny, warm day with a slight breeze and dry vegetation is the ideal weather to collect agents.
- **Peak emergence.** Adult weevils can be seen at times other than the stated optimal emergence dates. This is due to varying emergence times for each individual insect. For the greatest success in collecting the adult agents, however, collect the agents during the peak emergence period listed for each agent in Table 12.

- **Correct weed stage.** Adult agents die after their bud stage for oviposition is completed. They also move to higher elevations for the appropriate growth stage of yellow starthistle. Time the collection for the peak emergence period before that is a problem.
4. *Obtain containers for collection* - Obtain breathable containers for transportation. Suggestions for possible containers are as follows:
 - A small non-waxed paper pint ice cream carton works the best.
 - A container with organdy cloth glued into a hole on top or rubber banded in place for air.
 - Paper bags work if care is taken to keep the bag from water and heavy objects.
 - Do not use plastic bags because they will trap condensation and drown the insects.
 5. *Number of agents to collect* - The minimum number of agents per release needed to ensure establishment is 100 agents per release site; 150 to 200 agents per site are best. Generally, more is better.
 6. *Care of the agents* - Follow the guidelines in "How to Transport (and/or Ship) the Agents" to care for the agents as they are collected.
 7. *Record data on the Agent Release Form* - Record the collection date, collection site location (TRS or GPS), and the name and address of collector on the Agent Release Form (Appendix A).
 8. *Suggestions for optimal collections* – Remember the following when collecting agents:
 - **5-10% bloom.** A good time to work with the most agents at one time is when the yellow starthistle is at 5-10% bloom. One to two weeks earlier than 5-10% bloom for *B. orientalis*, *C. australis* and *U. sirunaseva*, then *E. villosus* and *L. curtus*.
 - **Heat of the day.** The best time of the day to collect agents is during the heat of the day between 1:00 and 6:00 p.m. because the agents are more active at that time.
 - **Dry weather.** For best agent survival results, collecting in the rain is not advised. The wet agents have a high mortality rate. If it is rainy or damp the day of collection (or even the day before), the success of the collection will decrease dramatically.
 - **Multiple collections.** Sweeping can knock heads off the yellow starthistle. This is a consideration when making multiple collections in one season at the same site.
- DO NOT PUT MORE THAN 200 AGENTS PER PINT CONTAINER.**
- PLACE AGENTS IN A COOLER AS SOON AS POSSIBLE. DO NOT ALLOW**

CONTACT BETWEEN THE AGENT CONTAINER AND THE BLUE ICE PACK.

9. Common mistakes

- **Rain.** Collecting in the rain.
- **Heat.** Exposing insects to extreme (>95°F) temperature.
- **Plastic bags.** Using plastic bags for containers. The condensation from the plastic bags damages the insects.
- **Low agent numbers.** Collecting at a new nursery site before site is established.
- **Time of day.** Collecting agents at the wrong time of day or yellow starthistle growth stage.
- **Trespassing.** Trespassing on private land.
- **Air.** Failure to provide ventilation in holding container.

There are three methods of collecting agents: sweep netting, hand picking, and tap. Choose the method best suited to the agent, the site and the resources available.

10. Sweep net collection method for weevils – The sweep netting method is recommended for collecting weevils because it is relatively easy and efficient, sanitary (no unwanted or unknown material collected and released), and it assures the identity and quantity of the agent being released. Follow the steps listed below:

- **Sweeping.** The sweep netting method works best in teams of two. One person gently sweeps the top half of the yellow starthistle buds using a 15" canvas hoop net. The second person counts agents into breathable containers for each release site.
- **Shade.** Take a cooler with blue ice into the field for the agents. Tape the blue ice to the inside of the cooler so that it does not roll around and crush the agents. Place crumpled newspaper between the blue ice and the agent container. While collecting, transporting and counting, keep the agents shaded and cool at all times.

DO NOT EXPOSE AGENTS TO THE SUN. KEEP SHADED AT ALL TIMES.

- **Remove debris.** Sort the agents so that unwanted materials such as weed seeds and potentially harmful organisms are not moved to the new site inadvertently.
- **Sort.** Since *E. villosus* will not fly off the tray, dump the contents of the sweep net onto a flat tray or leave the sweep net contents in the net and pick the weevils out of the debris by hand or tweezers and place them in a breathable container.
- *B. orientalis* and *L. curtus* will fly out of the net or off of a tray. One method is to grasp the net above the debris taking care not to crush agents, then slowly turn

the net inside out. Pick the weevils out of the debris as the net is turned. Another method is to aspirate the weevils out of the net as the net is opened.

- **Cover.** Keep a lid on the breathable container to keep the weevils from escaping.
- **Label.** Seal and label the carton with the species, number of agents, collection site and date.

11. *Sweep net collection method for flies* – This method is also recommended for flies for the same reasons stated above. Use the method listed above for weevils with the following changes and suggestions:

- **Sweep.** Sweep the air above the yellow starthistle buds using a canvas hoop net.
- **Shade.** Take a cooler with blue ice into the field for the agents. Tape the blue ice to the inside of the cooler so that it does not roll around and crush the agents. Place crumpled newspaper between the blue ice and the agent container. While collecting, transporting and counting keep the agents shaded and cool at all times.

DO NOT EXPOSE AGENTS TO THE SUN. KEEP SHADED AT ALL TIMES.

- **Sort.** One method is to shake the contents of the net into a large breathable container, to be placed immediately in the cooler. Separate the agents as soon as possible in the laboratory.
- **Aspirate.** Use an aspirator to suck the flies from the net or breathable container into the collecting jar. Aspirators can be purchased from a biological equipment source or constructed in the laboratory using the following materials (see Fig 31). The intake and exhaust tubes are copper tubing and the mouthpiece is amber latex tubing (ID 3/16"; OD 5/16"; wall diameter 1/16"). The 9-dram collecting vial is capped with a 5-1/2" two-hole rubber stopper. The exhaust tube is protected with fine 220-mesh netting to limit inhalation of foreign matter.

Aspirating the flies can be done in the field or in the laboratory. At the laboratory, place the flies in a cooler at 40°F to 50°F or a Plexiglas cage for ease of aspiration.

- **Label.** Seal and label the carton with the species, number of agents, collection site and date.
- **Mortality.** There can be some mortality in sweep netting flies because flies are very delicate. The best method is to sweep for 2 to 5 minutes, then aspirate. Alternate between sweeping and aspirating until the collection is complete. This reduces the potential harm to the agents by being knocked about in the net with debris and other insects.

DO NOT CONFINE THE FLY AGENTS IN THE SWEEP NET FOR LONG PERIODS OF TIME.

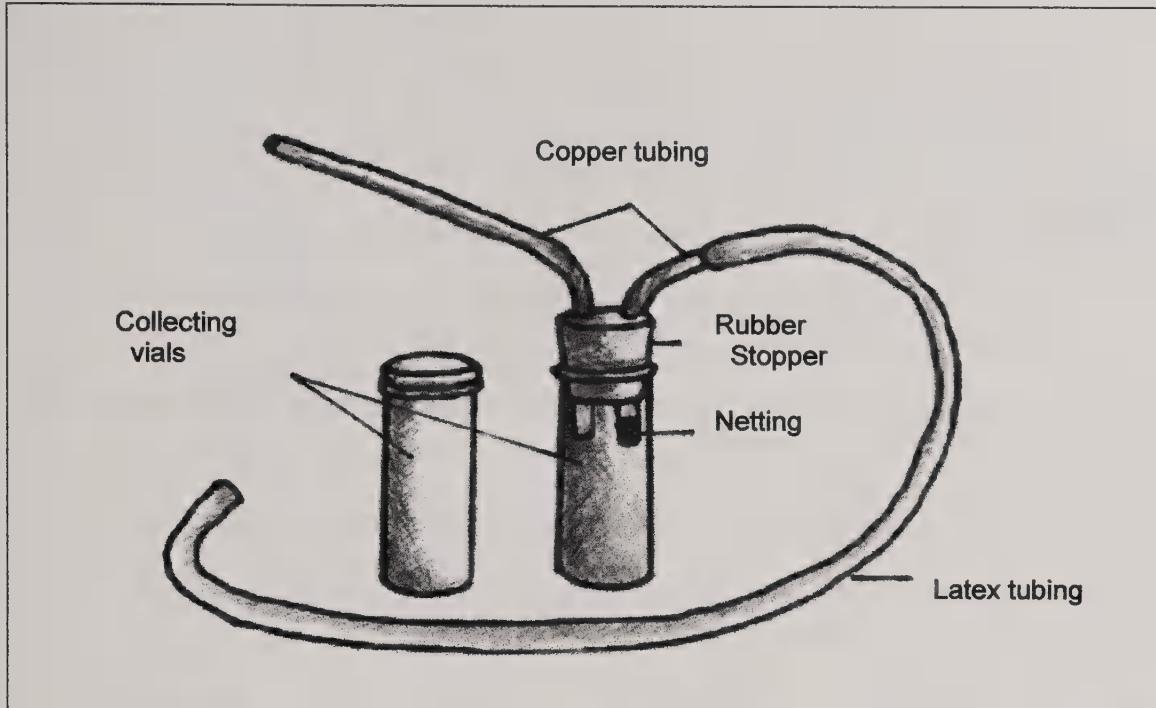


Figure 31. The aspirator is used to capture insects that may be damaged with forceps.

12. **Handpicking collection method** – Weevil agents can be handpicked from the yellow starthistle plant.

- **L. curtus.** This method works very well for *L. curtus* as the agents are found in the flowers with their hind end sticking up in the air. Pluck the agent gently out of the flower head. Place the weevil immediately into in a small container. When 5-10 weevils have been collected in the small container, transfer into a larger container with the other weevils. Repeat the process until the larger container is filled with weevils (less than 200).
- **Funnel.** A funnel aids in trapping the insects into the collection container. A plastic pop or juice container can be easily converted into a funnel by cutting in half, inverting the small neck into the bottom and taping them together. However, ventilation must be provided (such as netting over the bottom) to prevent agent mortality.
- **Shade.** Take a cooler with blue ice for the agents into the field. While collecting, transporting and counting agents, maintain shade for the agents at all times.

DO NOT EXPOSE AGENTS TO THE SUN. KEEP SHADED AT ALL TIMES.

- **Contraindications.** With the exception of collecting *L. curtus*, this method is labor intensive and is not useful for collecting flies due to their fragility.

13. **Tap collection method** - This is the easiest method for weevils if a net is unavailable.

- **Tap the stalk.** Use a stick (a badminton racquet works very well) to gently tap the stalk of the yellow starthistle plant over a bucket to knock the agent into the bucket. Separate the agents from unwanted material and place in a breathable container.

REMEMBER THAT *E. VILLOSUS* TENDS TO CLING TO THE PLANT MATERIAL.

- **Shade.** Take a cooler with blue ice for the agents into the field. While collecting, transporting and counting, maintain shade for the agents at all times.

DO NOT ALLOW CONTACT BETWEEN THE AGENT CONTAINER AND THE BLUE ICE PACK.

DO NOT EXPOSE AGENTS TO THE SUN. KEEP SHADED AT ALL TIMES.

- **Contraindications.** Since flies hover over the plant and will fly away when disturbed, this method is not useful for flies.

14. **Collection methods specific to each agent** - Use the Table 12 when collecting agents.

Table 12. Collection Methods Specific to Each Agent

Agent	Method
<i>Bangasternus orientalis</i>	The adults are inactive and difficult to see during the cool morning hours, however, sweeping at any time during the day will be successful if the weevils are present. The tap method is an efficient and productive collection method for collecting <i>B. orientalis</i> , although sweep netting is also recommended.
<i>Eustenopus villosus</i>	Sweep netting is the recommended method. When collecting <i>E. villosus</i> , it is helpful to remember that it tends to cling tightly to the plant.
<i>Larinus curitus</i>	<i>L. curitus</i> is easily found with their hind end up in the yellow starthistle flower. Handpicking or tap method works well. Gently pluck, tap or scrape the agent from the flower head into a small vial, keeping the lid in place to keep weevils from escaping. Place in a breathable container when 5-10 agents are collected.
<i>Chaetorellia australis</i> <i>Urophora sirunaseva</i>	Adult flies can be collected by sweeping, but it is difficult and the flies can be damaged. The seed head collection method is the easiest.

How to Release Adult Bioagents

After collecting the adult agents, the next step is to release the agents at the selected site. Follow these steps:

- 1. Gather supplies**
 - 2. Determine when to release the agents**
 - 3. Transport the agent**
 - 4. Release the adult agents**
 - 5. Release methods for each agent**
 - 6. Voucher specimens**
 - 7. Release form**
 - 8. Frequency of release**
 - 9. Releasing multiple agents**
 - 10. Suggestions for optimal establishment**
 - 11. Common mistakes**
-

1. *Gather supplies* – Prepare the following supplies to take into the field:
 - Pencil
 - Agent Release Form
 - Release cage for flies (optional)
 - Photo point supplies (optional)
2. *Determine when to release the agents* – To decide when to release the agents consider the following:
 - **Bud stage.** Release the agent when the yellow starthistle plant is in the appropriate bud stage for the agent being released as listed in Tables 5 and 6.

RELEASE THE AGENTS AS SOON AS POSSIBLE WITHIN 24 HOURS OF COLLECTION, OR RECEIPT IF ORDERING BY MAIL.

 - **Reference.** Check with the county extension agent or county weed supervisor.
 - **Contraindications.** If most yellow starthistle buds are beyond the recommended release stage, it is too late to release at that site.
3. *Transport the agent* – After collection, transport the agents to the release site using the following suggestions for optimal survival of agents:

- **Background.** Read and follow the section on “How to Transport the Agents.”
- **Ice chest.** Transport the container of agents to the field in a cooler with a blue ice pack.

Tape the blue ice in the bottom of the cooler. To protect the agents from freezing, put crumbled newspaper on top of the blue ice. Place the agent container on top of the newspaper.

DO NOT ALLOW CONTACT BETWEEN THE AGENT CONTAINER AND THE BLUE ICE PACK.

4. *Release the adult agents* – Release the agents at the site as soon as possible after collecting, receiving or rearing.
 - **Weather.** Do not wait for good weather. If you must release in the rain, provide shelter for the agents until they can disperse on their own. One way to do this is to place a cardboard box on its side, place the container in the box and open the lid. The agents will disperse when the weather conditions improve.
 - **Placement.** When releasing adult weevils or flies, place the agents on the ground within a 3 foot radius of the permanent location marker under yellow starthistle plants where they can continue to mate and disperse on their own.
5. *Release methods for each agent* - Use Table 13 for release methods specific to each agent.
6. *Voucher specimens* – Retain voucher specimens at each release site. The specimens are a means of identifying and recording for future reference the agent(s) released.
 - **Specimens.** Retain 5-10 agents (dead or alive) for voucher specimens. Put in a small vial with 70% ethyl alcohol for a voucher specimen.
 - **Label.** Write in pencil the name of the agent, source of the agent, the date of release, person or agency releasing and release site on a piece of paper and place inside the vial. **Always use a pencil as the alcohol will dissolve and bleed ink from pens and markers.**
 - **In-House records.** Save several specimens for in-house records.
 - Mail voucher specimens to:
Frank Merikel
University of Idaho
Department of Plant, Soil and Entomological Sciences
Moscow, ID 83844-2339
7. *Release form* – Fill out the Biological Control Agent Release Form (Appendix A).

Table 13. Release Methods for Each Agent

Agent	Method
<i>Bangasternus orientalis</i>	Release mating weevils during the BU-1 through BU-3 bud stages.
<i>Eustenopus villosus</i>	Release mating weevils during the BU-3 and BU-4 bud stages. Releasing <i>E. villosus</i> adults in a grid pattern 100-feet apart is a way of yielding good blanket coverage of <i>E. villosus</i> at a site. <i>E. villosus</i> tends to disperse more readily uphill than downhill. Releasing the agents at the bottom of the hill encourages the agents to follow the phenological stages of the yellow starthistle uphill, which may increase the rate of spread.
<i>Larinus curitus</i>	Release mating weevils during the flower stage.
<i>Chaetorellia australis</i> <i>Urophora sirunaseva</i>	Release mating <i>C. australis</i> during the BU-3 bud stage. Release mating <i>U. sirunaseva</i> during the BU-2 and BU-3 bud stages. Place the flies in the field in the early morning at ground level beneath yellow starthistle plants. The flies are rapid dispersers. The cool morning hours allow the flies to mate and adjust to their environment before moving about. To optimize success, a release cage is recommended for releasing flies. Build a simple cage tall enough and wide enough to enclose several yellow starthistle plants. Cover the frame with organdy cloth or 100-mesh screen. Or purchase a cage from a biological supplier. Place the flies in the cage. After 24 hours remove the cage. This method allows the agent to mate and adjust to the environment before moving about.

8. *Frequency of release* – Release agent once per site per season.

- **Successive releases.** Successive releases may be appropriate depending on previous success of release. Make the releases at different sites starting approximately 0.5 mile from a previous release site.
- More extensive releases over greater distances are best following local success.
- **Augmentative releases.** Releasing additional biological control agents in an area where they are already present (augmentative releases) is not an efficient strategy when agents are not available in large numbers or already well distributed.

9. *Releasing multiple agents* - Releasing too much genetic variation may have negative effects.

- **Evaluate.** The best practice is to evaluate populations and sites and select the agent which best fits its respective situations.
- **Separate species.** As a guideline, separate the species by at least 100m so that species establishment will not be impeded by the activity of another species.

It is expected that eventually agent populations will overlap and sort themselves out naturally depending on the habitat, population and weed levels.

10. Suggestions for optimal establishment

- **Number.** A minimum of 100 agents needs to be collected for each release. A release of 200 agents is optimum.
- **Time of day.** Releasing in the early morning hours between 6:00 and 10:00 a.m. or in the cooler evening hours between 3:00 and 9:00 p.m. is recommended.
- **Weather.** Avoid releasing agents during rainy or very hot weather for optimal establishment. However, sometimes it may be necessary to release in the rain.
- **Bottom of hill.** Releasing weevil agents at the bottom of the hill encourages them to follow the phenological stages of the yellow starthistle uphill, and may increase the rate of spread, however, there is no data to support this observation.
- **Care.** Common sense and care is a major factor in the survival and establishment of the insects.

11. Common mistakes – Here are some common mistakes made in releasing agents.

- **Weed stage.** Yellow starthistle is not at the proper stage. The agents need to oviposit on the correct bud or flower stages. The lack of appropriate bud or flower stages available at the site for the agent for reproducing suggests that the release would be unsuccessful.
- **Transportation.** Agents die in transit.
- **Rain.** Releasing in rainy weather.
- **Documentation.** Not documenting release site information, especially location. Make maps to release sites and take photographs for future reference.

How to Transport Bioagents

Follow these steps to transport agents from the collection site:

- 1. Gather supplies**
 - 2. Label the container**
 - 3. Keep agents cool**
 - 4. Avoid exposure to sun**
 - 5. Delayed distribution**
 - 6. Immediate distribution**
 - 7. Common mistakes**
-

1. *Gather supplies* - Prepare the following supplies:

- Breathable containers
- Damp sponge
- Tape
- Paper toweling or toilet paper
- Ice chest
- Blue ice pack

2. *Label the container* - Seal the breathable container with tape, making certain that agents are not exposed to the tape. Label the container with the name of the agent, the amount of agents collected and the collection date.
3. *Keep agents cool* - Place the blue ice pack in the bottom of the cooler. Crumple a layer of newspaper over the ice and place the agent container on top of the newspaper. Tape down the blue ice container to avoid physical damage to the insects. Protect the agents from water or excess moisture by placing a barrier between the agents and the moisture source. An alternative is to refrigerate at 40°-50° F (4°C) as soon as possible to keep insects cool.

DO NOT ALLOW CONTACT BETWEEN AGENT CONTAINER AND THE BLUE ICE PACK.

MAKE CERTAIN INSECTS ARE WELL PROTECTED. INSECTS WILL NOT TOLERATE WATER AND HEAVY OBJECTS.

4. *Avoid exposure to sun* - Avoid exposing biocontrol agents to heat or direct sunlight.

DO NOT LEAVE THE CONTAINER IN THE SUN AT ALL BUT ESPECIALLY NOT ON THE DASHBOARD OR SEAT OF THE VEHICLE.

5. *Immediate distribution* - For immediate distribution, store in a breathable container with fresh yellow starthistle stems that are in the appropriate bud stage or one inch of slightly damp sponge or paper towel (the sponge must contain no dyes or phosphates). Wring out the sponge or paper towel *thoroughly*. WHEN USING VEGETATIVE MATERIAL DO NOT INCLUDE ANY FLOWERS, SEEDS, OR ROOTS.

DISTRIBUTE THE AGENTS AS SOON AS POSSIBLE, WITHIN 24 HOURS IS BEST.

6. *Delayed distribution* - If waiting to release cannot be avoided, the insects can be stored up to three days in a refrigerator, but no longer than one day is recommended. For storage longer than one day, follow the guidelines in "Keeping Bioagents Alive."
7. *Common mistakes*
 - **Excess heat.** Putting agents on the vehicle dashboard or in the sun.
 - **Water.** Drowning agents in spilled or melted water.
 - **Air.** Container not breathable.

How to Ship Bioagents

Follow these steps for shipping agents after collection:

- 1. Gather supplies**
 - 2. Obtain regulations**
 - 3. Remove possible contamination**
 - 4. Shipping containers**
 - 5. Supply agent with food and water**
 - 6. Tape lids**
 - 7. Pack the container**
 - 8. Shipping time**
 - 9. Common mistakes**
-

1. Gather the following shipping supplies:

- Pen or pencil
- Breathable shipping container
- Newspaper or paper towel
- Food and water for agents
- Tape
- Ice chest
- Blue ice

- 2. Obtain regulations** - If it is necessary to ship agents out of the country or state, contact the local weed district, cooperative extension agent, or the US Department of Agriculture for regulations and restrictions.
- 3. Remove possible contamination** - Remove all unwanted material from the container to avoid contamination at the receiving site. The container will contain only the agent and its food source.
- 4. Shipping containers** - Use containers that will allow the agents to move about within the container. Provide the agents with something to walk on such as wadded up paper towel. If the agents are trapped on their backs, they may die.

5. *Supply agent with food and water* - Provide food, air and water while in transit. If using yellow starthistle plant materials as a food source do not ship roots and seeds. See "Keeping Bioagents Alive" for more information about feeding agents.
6. *Tape lids* - Tape lids on the breathable containers. Make sure the agents are not exposed to the tape or they will get stuck and die.
7. *Pack the container* - Pack the agents carefully so that they are not damaged during shipping and keep agents cool until released. Ice chests with 'blue ice' blocks taped to the sides and packed with a layer of paper to absorb the condensation work well for shipping.

DO NOT ALLOW CONTACT BETWEEN AGENT CONTAINER AND THE BLUE ICE PACK.

8. *Shipping time* - Avoid undue stress on the agents by planning the route and timing of the shipment of the insects with the shipping agent. Ship the agents quickly, the same day if possible, but at least within 24 to 48 hours of collection.
9. *Common mistakes* – Here are the common mistakes made in shipping agents.
 - Drowning
 - Asphyxiation
 - Insects get wet or smashed
 - Too many hours in transit or storage

How to Keep Bioagents Alive

The agents can be kept alive by supplying with food and water while in the release cage or during transportation or shipping following these steps:

- 1. Gather supplies**
 - 2. Supply agent with water**
 - 3. Feed the agents**
 - 4. Care for the agents**
 - 5. Store the agents**
-

1. Gather the following supplies:

- Small-hole phosphate and dye free sponge
- Cotton wicking (optional)
- Honey/water or sugar/water solution
- Refrigerator or ice chest

2. Supply agents with water – Use the following suggestions for providing the agents with water:

- Use a natural sponge with small holes and no dye or phosphate content. Chemicals in the sponge can be harmful to the agents. Sponges with large holes are death traps and drown the agents. To prevent drowning, make sure there is no free standing water. Wet a sponge, wring out the water and place the damp sponge in a same-sized container.
- Alternatively, use a length of dental cotton wicking stuck into a narrow-necked container of water. Plug the hole with cotton wicking so that no open water is available. Make sure that the water container does not tip or spill.
- Check water daily.
- Keep the agents dry.

3. Feed the agents – Feed the agents in one of the following ways:

- **Sponge method.** The best method is to glue a piece of sponge dampened with sugar solution or soda pop like Mountain Dew to the top or sides of the container and moisten as needed.

- **Pin method.** Dip a pin (or needle or the stiff hair from a broom) into a sugar solution and make 3 or 4 light stripes across the top of the Plexiglas cage or sides of the plastic carton.
- **Spray method.** Spray or lightly mist the top or sides of the container with a spray bottle. Do not spray the flies directly as it can bond their wings to their body.
- Make the sugar solution light, almost dry, sticky line or spot, not wet or dripping. Check as often as necessary depending on the number of agents in the cage. Reapply when no lines or spots are visible.
- Fresh yellow starthistle stems and the appropriate bud stage buds can also be used for feeding weevils only during travel or storage. This method is contraindicated for flies as the plant material gets knocked around and damages the flies. Take care not to transport any part of the yellow starthistle plant that is capable of reproducing (such as roots or seeds) to another site.

Sugar Solution

	Pin Method	Spray Method
Honey/water	1:1	2:1
Sugar/water	2:1	4:1

4. *Care for the agents* – Follow these guidelines to care for the agent until release:
 - Provide something for the agents to crawl around on, such as wadded up paper toweling, toilet paper or plant stems.
 - Avoid physical damage to the agent by taping down potentially harmful objects, such as blue ice packs.
 - Ensure that predators are not trapped inside the containers with the agent.
 - Provide adequate ventilation for the agent.
 - Avoid exposing agent to excessive heat.
5. *Store the agents* – Agents can be refrigerated for several weeks if absolutely necessary.
 - Provide intermittent periods of warm temperatures to allow feeding at least 2 times per week for 2 hours.
 - Store briefly in refrigerators no colder than 40°-50° F (4°C) or in cool ice chests until shipment or transfer.

How to Collect Seed Heads for Releasing Flies

Flies are fragile and can be damaged easily in the process of collecting, transporting and releasing. Collecting adult flies is also more labor intensive and prone to failure. Collecting yellow starthistle seed heads for placement in the field or rearing adult flies in the laboratory is an alternative to collecting adult flies for release. Follow these steps:

- 1. Gather supplies**
 - 2. Subsample the seed heads**
 - 3. Count the number of agents in the subsample**
 - 4. Estimate sample size from subsample**
 - 5. Determine when to collect the seed heads for fly release**
 - 6. Collect the seed heads for fly release**
-

- 1. Gather supplies** – Prepare the following supplies to take to the site:
 - Gallon size re-sealable plastic bag
 - Clippers
 - Gloves
 - Data sheet
 - Pencil(s)
 - Wide rubber bands
 - Sharpie
 - Clear container for fly emergence
- 2. Subsample the seed heads** – Since the extent of agent infestation at the collection site is unknown, the quantity of stems to collect is also unknown. Subsampling is used to determine the number and species of individual agents present in the seed heads and the amount of material to collect for release.
 - Inspect the collection site to confirm that fly larvae are present. Infested seed heads usually have some of the old bracts or florets attached when infested with *C. australis*. *U. sirunaseva* galls are easily felt in the old seed head buttons.
 - Collect the seed heads in March when the flies are in diapause. This is important because otherwise the flies will not emerge in a timely fashion.
 - Collect seed heads for the subsample using the following steps:

- ✓ Locate the permanent location marker at the collection site. If monitoring at a site with no previous release, mark a temporary point with a stake or clipboard.
 - ✓ Collect two seed heads per plant at random along four lines in N, S, E and W directions from the permanent location marker.
 - ✓ Collect 50 seed heads from each line for a total of 200 seed heads.
 - ✓ Place the seed heads in a paper bag.
 - ✓ Label the bag with the site name and date for future identification.
3. Count the number of agents in the subsample – The two methods of estimating population and species composition are the rearing of adult flies and the dissection of the seed heads.
- Rearing adult flies method. Do the following steps to rear adult flies for release.
 - ✓ Empty fly-infested seed heads from the collection bag to a clear, breathable container such as a covered terrarium or covered petri dish.
 - ✓ Place flies in a sunny (but not hot) spot inside a building.
 - ✓ Flies will begin to emerge from the seed heads in 2-3 weeks.
 - ✓ Keep bags for at least 8 weeks from the date of original emergence and 2-4 weeks beyond the last fly emergence.
 - ✓ When fly emergence has stopped, count and record the number and species of flies that emerge from each sample.
 - ✓ Dissect seed heads for further evidence of fly activity. Look for pupal cases, dried out larva, etc. Record how many seed heads were infested.
 - Dissection method. It is impractical to distinguish between the two species of fly larvae and pupae; however, a combined proportion of infested seed heads can be determined. Follow these steps when using this method:
 - ✓ Dissect the seed heads immediately after collection.
 - ✓ Count and record the number of fly larvae or pupae found in 200 seed heads.
4. Estimate sample size from subsample – Calculate how many bouquets are needed to collect a minimum of 150 flies by using the following equation.
- Estimated number of seed heads per bouquet (800 seed heads) × the number of larvae/galls per seed head = the number of flies per bouquet. The number of flies per bouquet ÷ the number of seed heads needed to release at a site (minimum of 150) = number of bouquets of yellow starthistle needed for a release.

- Example: 800 seed heads x an average of 2 *Urophora* fly galls per seed head = 1600 *Urophora* flies per bouquet. Ten bouquets make one release.
5. *Determine when to collect the seed heads for fly release.* Collect the seed heads in March or April after the flies have completed diapause and just before they emerge because the aganet needs to have a certain amount of cumulative cold days to complete its normal cycle. Waiting to collect until the right before emergence also reduces the amount of storage time.
- ✓ Seed heads can also be collected in the winter (January-March), stored outside in a barn or shed to complete their diapause, and then brought inside in March or April to emerge.
 - ✓ Larvae collected in seed heads in the fall often desiccate before their emergence in the spring, therefore collecting later in the winter and spring is more successful.
6. *Collect the seed heads for fly release* – After determining collection size, collect the seed heads in the following manner.
- Collect two seed heads per plant along four lines in N, S, E and W directions from the permanent location marker.
 - Collect 50 seed heads from each line for a total of 200 seed heads.
 - Place them in the paper bags as collected.
 - Label the bags with the site name and date for future identification.
 - Collect the number of seed heads as determined in the results of the subsampling effort listed above.
 - Record location information on a data sheet for future reference.
 - Store at a constant temperature between 36-40° F until ready to release.

How to Release Fly Infested Seed Heads

After the seed heads have been collected, decide the best method of releasing the flies at the site. Follow these steps:

- 1. *Gather supplies***
 - 2. *Placement method***
 - 3. *Release cage method***
 - 4. *Rearing method***
-
-

1. *Gather the following supplies:*

- Rubber bands
- Tarp (optional)
- Wire or string
- Release cage (optional)
- Large (13 gallon) plastic bags

2. *Placement method* - In this method the seed heads are placed on the release site and left to develop. The flies emerge, feed and reproduce.

- Break off last year's plants and develop bouquets as large in diameter as the hand will hold.
- Hold the bouquet together with a wide rubber band on the stem part of each bouquet to hold it together.
- Make enough bouquets for the release(s).
- Load the bouquets into a covered vehicle or a tarp or other barrier to prevent the seed from spreading.
- Place them at the release site before the bolting stage. Secure one bouquet per post, stake or fence post.
- Advantages in using the placement method.
 - ✓ Placing yellow starthistle seed heads at the release site is the easiest method for releasing flies.
- Disadvantages in using the placement method.

- ✓ Noxious weed seeds can be inadvertently transferred to the release site.
- ✓ There is potential to introduce new strains of yellow starthistle to the release site, such as herbicide resistant yellow starthistle.
- ✓ There is no guarantee that the flies will survive.
- ✓ There is no way to determine how many seed heads actually contain larvae, therefore no way to determine the exact amount of flies released.
- ✓ There is no way to determine whether larvae were parasitized.
- ✓ There is no way to determine which fly is present.
- ✓ There is a potential problem with rodents feeding on the seed heads at the release site and reducing the potential number of agents released with the placement method.
- ✓ The greater the distance between the collection site and the release site, the greater the risk of contamination. Collection sites within five miles of the release site are considered reasonably safe.

3. *Release cage method* - An alternative to placing the seed heads at the release site is to place the seed heads in a release cage (Fig. 32).

- Store the release cage with the seed heads in a protected area, such as a barn. This is necessary to prepare the insects for normal development in the spring.
- Move the cages to the release site any time in early spring before the yellow starthistle bolting stage. The adult flies will emerge and escape from the cage in the spring when warmer temperatures allow them to complete their development.
- The advantage to using the release cage method is that the release cage helps prevent the spread of seeds that may have remained in the seed heads.



Fig. 32. Release cage for containing seed heads during bioagent release.

4. *Rearing method* – The flies are reared in the laboratory with this method.

- Gather the following supplies for rearing agents:
 - ✓ Large (13 gallon) plastic garbage bags
 - ✓ Cold storage

- ✓ Aspirator
- ✓ Rearing container
- ✓ Organdy cloth
- Keep seed heads in cold storage (35-40° F) until 4-5 weeks prior to normal emergence of the agent. Place in the rearing container at room temperature and mist seed heads with water every day.
- Monitor for straw itch mites that may contaminate colonies.
- Aspirate the flies from the container every day for two weeks and place at the release site as soon as possible. Maximum holding time for the flies is 3-4 days.

DO NOT PUT MORE THAN 100 FLIES IN EACH CONTAINER.

- Use the guidelines in "Keeping Bioagents Alive" to care for the flies while storing.
- When rearing flies in the laboratory, note that a 24-hour-old fly is at a perfect stage to release; however, flies can be kept for 3-4 days until release.
- Advantages in using the rearing method.
 - ✓ Agents collected in dry plant material can be reared in the laboratory until they can be identified, sorted and then released in the field as adults.
 - ✓ Rearing flies guarantees the identity and quantity of the agent to be released and is sanitary, however, it is more labor, space and equipment intensive.
 - ✓ This method will eliminate problems of physical damage to the fly during sweeping and rodent feeding on the seed heads in the field.
 - ✓ It will also screen out unwanted material that could potentially be transferred to the release site, i.e. noxious seeds, insect pests or pathogens.
- Rearing container. The rearing container may be of any design or dimension as long as it is large enough to hold the dry plant material, confine the agent and provide air, food and water until it is released. Following are two examples of rearing containers:
 - ✓ Construct a container with a window (Plexiglas™) with 2 sleeves (muslin or organdy cloth), hinges to open the window, a screen back (organdy cloth or 200 mesh screen) (see Fig. 33). The dimensions are completely arbitrary according to need. They can also be ordered in different sizes from a biological equipment source.

✓ Use a plastic cottage cheese/yogurt/any similar container with a tight fitting lid for a rearing chamber. Use any size necessary to hold the number of seed heads available. Cut a hole in the plastic top for air. Either glue organdy cloth to the top over the hole, or rubber band the organdy cloth over the bottom container and secure the cloth in place with the container top (with the hole cut in the middle).

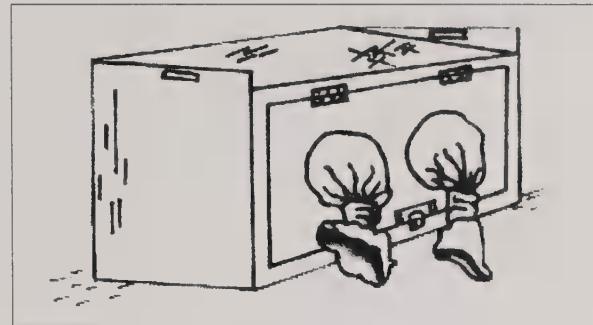


Fig. 33. Rearing container for rearing bioagents in the laboratory.

Bibliography

Biological Control

Antognini, J., P.C. Quimby, Jr., C.E.Turner and J.A.Young. 1995. Implementing effective noxious range weed control on rangelands. *Rangelands* 17(5): 158-163.

Attavian, Bruce N. 1995. Biological control of diffuse and spotted knapweed. USDA, APHIS, PPQ. Fredericks, MD.

Attavian, Bruce N. 1994. Biological control of leafy spurge project manual. USDA, APHIS, PPQ. Fredericks, MD.

DeBach, P. and D. Rosen, eds. 1991. Biological control by natural enemies. 2nd ed. Cambridge Univ. Press, Cambridge and New York.

Harley, K.L.S. and I.W. Forno, eds. 1992. Biological control of weeds: a handbook for practitioners and students. Inkata Press, Melbourne and Sydney.

Harmon, B.L. and J.P. McCaffrey. 1989. Biological control of weeds in Idaho: bioagent release records. Univ. of Idaho, College of Agric., Current Information Series No. 707.

James, L.F., J.O. Evans, M.H.Ralphs and R.D. Child, Eds. 1991. Noxious range weeds. Westview Press, Boulder CO.

Pedigo, L. P. 1996. Entomology and pest management. 2nd Edition. Prentice-Hall Inc., N. J.

Randall, J.M. 1996. Weed control for the preservation of biological diversity. *Weed Technol.* 10: 370-383.

Rees, N.E., P.C. Quimby, Jr., G.L. Piper, E. Coombs, C.E. Turner, N.R. Spencer and L.V. Knutson, eds. 1996. Biological control of weeds in the west. Western Society of Weed Science.

Sheley, R.L., T.J. Svejcar and B.D. Maxwell. 1996. A theoretical framework for developing successional weed management strategies on rangeland. *Weed Technol.* 10: 766-773.

Van den Bosch, R., P.S. Messenger and A.P. Gutierrez. 1982. An introduction to biological control. Plenum Press, N.Y.

Van Driesche, R.G. and T.S. Bellows, Jr. 1996. Biological Control. Chapman and Hall, N.Y.

Whitson, T.D., L.C. Burrill, S.A. Dewey, D.W. Cudney, B.E. Nelson, R.D. Lee and R. Parker, eds. 1991. Weeds of the west. Western Society of Weed Science, Laramie, WY.

Yellow starthistle (*Centaurea solstitialis* L.)

Callihan, R.H., F.E. Northam, J.B. Johnson, E.L. Michalson and T.S. Prather. 1989. Yellow starthistle: biology and management in pasture and rangeland. Univ. Idaho College of Agric. Coop. Ext. Serv. Curr. Info. Ser., No. 634.

Callihan, B., L. Smith, J. McCaffrey, and E. Michalson. 1995. Yellow starthistle management for small acreages. Univ. Idaho, College of Agric, Current Info. Series No. 1025.

Callihan, R.H., T.S. Prather, and F. E. Northam. 1993. Longevity of yellow starthistle (*Centaurea solstitialis*) achenes in soil. *Weed Technol* 7: 33-35.

Clement, S. L. 1990. Insect natural enemies of yellow starthistle in southern Europe and the selection of candidate biological control agents. *Environ. Entomol.* 19:1882-88.

Cordy, D.R. 1978. *Centaurea* species and equine nigropallial encephalomalcia. In: Effects of poisonous plants on livestock. Keeler, R.F. (Eds: van Kampen, K.R. and L.F. James)(eds.) Academic Press, New York, 327-336.

Higgins, R.E. and R.L. Kambitsch. 1978. Yellow starthistle. A threat to Idaho land users. Univ. Idaho, College of Agric., Current Information Series No. 445.

Joley, D.B., D.M. Maddox, D.M. Supkoff and A. Mayfield. 1992. Dynamics of yellow starthistle (*Centaurea solstitialis*) achenes in field and laboratory. *Weed Sci.* 40: 190-194.

Lanini, W.T., C. Thomsen, T.S. Prather, C.E. Turner, M.J. Smith, C.L. Elmore, M. Vayssieres and W.A. Williams. 1994. Yellow starthistle. Univ. Calif. Div. Agric. Nat. Res., Pest Notes No. 3.

Maddox, D.M. 1981. Introduction, phenology, and density of yellow starthistle in coastal, intercoastal, and central valley situations in California. USDA-ARS ARR-W-20.

Maddox,D., R. Sobhian, D.B. Joley, A. Mayfield and D.Supkoff. 1986. New biological control for yellow starthistle. *Calif. Agric.* 40 (11 and 12):4-5.

Roché, B.F.,Jr. 1992. Achene dispersal in yellow starthistle (*Centaurea solstitialis* L.). *Northwest. Sci.* 66: 62-65.

Rosenthal, S.S., G. Campobasso, L. Fornasari, R. Sobhian and C.E.Turner. 1991. Biological control of *Centaurea* spp. pp. 292-302. In: James, L.F., J.O. Evans, M.H. Ralphs and R.D. Child, eds. Noxious Range Weeds. Westview Press, Boulder, CO.

Ross, E. 1981. 1981 survey of yellow starthistle (*Centaurea solstitialis*) infestations in Idaho. 30 pp.

Sheley, R.L. and L.L. Larson 1994. Comparative growth and interference between cheatgrass and yellow starthistle seedlings. *J. Range Manage.* 47: 470-476.

Sheley, R. and L. Larson. 1995. Interference between cheatgrass and yellow starthistle at 3 soil depths. *J. Range Manage.* 48: 392-397.

Thomsen, C. D., W. A. Williams, M. P. Vayssières, C. E. Turner and W. T. Lanini. 1996. Yellow starthistle biology and control. University of California. Publication 21541.Woods, D. M., (ed.) 1997. Biological Control Program Annual Summary, 1996. California Department of Food and Agriculture, Division of Plant Industry, Sacramento, California.

Thomsen, C.D., W.A. Williams, M.R. George, W.B. McHenry, F.L. Bell and R.S. Knight. 1989. Managing yellow starthistle on rangeland. *Calif. Agric.* 43(5): 4-7.

Turner, C.E. 1994. Biological control of yellow starthistle: 1994 update, pp. 224-227. In: Proceedings California Weed Conference. 17-19 January, 1994. San Jose, California.

Turner, C.E. 1991. Biological control of yellow starthistle, *Centaurea solstitialis* L.: a progress report, pp. 78-82. In: Proceedings California Weed Congress. 21-23. 1991. Santa Barbara, California.

Turner, C.E., J.B. Johnson and J.P. McCaffrey. 1994. Yellow starthistle *Centaurea solstitialis* L. (Asteraceae). In: Biological Control in the U.S. Western Region: Accomplishments and Benefits of Regional Research Project W-84 (1964-1989). University of California, Division of Agriculture and Natural Resources.

Bangasternus orientalis

Clement, S. L. 1990. Insect natural enemies of yellow starthistle in southern Europe and the selection of candidate biological control agents. *Environ. Entomol.* 19:1882-88.

Maddox, D.M. 1985. A petition for the importation into quarantine and subsequent release in the United States, of the weevil *Bangasternus orientalis* Capiomont (Coleoptera: Curculionidae), a candidate natural enemy for the biological control of yellow starthistle (*Centaurea solstitialis* L.).

Maddox, D.M., D.B. Joley, A. Mayfield and B.E. Mackey. 1991. Impact of *Bangasternus orientalis* (Coleoptera: Curculionidae) on achene production of *Centaurea solstitialis* (Asterales: Asteraceae) at a low and high elevation site in California. Environ. Entomol. 20: 335-337.

Maddox, D. M., and R. Sobhian. 1987. Field experiment to determine host specificity and oviposition behavior of *Bangasternus orientalis* and *Bangasternus fausti* (Coleoptera: Curculionidae), biological control candidates for yellow starthistle and diffuse knapweed. Environ. Entomol. 16: 645-648.

Maddox, D.M., R. Sobhian, D.B. Joley, A. Mayfield and D. Supkoff. 1986. New Biocontrol for yellow starthistle. Calif. Agric. 40(1&2):4-6.

Sobhian, R., and H. Zwolfer. 1985. Phytophagous insect species associated with flower heads of yellow starthistle (*Centaurea solstitialis* L.). Z. Angew. Entomol. 99:301-321.

Sobhian, R. G. Campobasso and P.H. Dunn. 1992. Contribution to the biology of *Bangasternus orientalis* Capiomont (Col., Curculionidae). J. Appl. Entomol. 113:93-102.

Chaetorellia australis

Clement, S. L. 1990. Insect natural enemies of yellow starthistle in southern Europe and the selection of candidate biological control agents. Environ. Entomol. 19:1882-88.

Maddox, D.M.; A. Mayfield and C.E. Turner. 1990. Host specificity of *Chaetorellia australis* (Diptera, Tephritidae) for biological control of yellow starthistle (*Centaurea solstitialis*, Asteraceae). In: Proceedings of the Entomological Society of Washington. 92: 426-430.

Pittara, I.S. and B. I. Katsoyannos. 1990. Evidence for a host-marking pheromone in *Chaetorellia australis*. Entomol. Exp. Appl. 54: 287-295.

Pittara, I.S. and B.I. Katsoyannos. 1991. Oviposition behavior, longevity, fecundity and fertility of *Chaetorellia australis* Hering (Diptera, Tephritidae). Bulletin De La Société Entomologique Suisse 64: 59-66.

Turner, C.E., G.L. Piper and E.M. Coombs. 1996. *Chaetorellia australis* (Diptera: Tephritidae) for biological control of yellow starthistle, *Centaurea solstitialis* (Compositae), in the western USA: establishment and seed destruction. Bull. Entomol. Res. 86: 177-182.

Eustenopus villosus

Clement, S. L. 1990. Insect natural enemies of yellow starthistle in southern Europe and the selection of candidate biological control agents. Environ. Entomol. 19:1882-88.

Clement, S.L. and R. Sobhian. 1991. Host-use patterns of capitulum-feeding insects of yellow starthistle: results from a garden plot in Greece. Environ. Entomol. 20(2):724-30.

Clement, S.L. and T. Mimmocchi. 1988. Occurrence of selected flower head insects of *Centaurea solstitialis* in Italy and Greece. Proc. Entomol. Soc. Wash. 90:47-51.

Connell, J. F. 1997. *Eustenopus villosus*: Its phenological synchrony with *Centaurea solstitialis* and its feeding and oviposition behavior. M.S. Thesis, University of Idaho.

Fornasari, L. and C. E. Turner. 1989. Petition for introduction and release in the United States of the weevil *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae) for biological control of yellow starthistle (*Centaurea Solstitialis* L.) (Asteraceae: Cardueae).

Fornasari, L., C.E. Turner and L.A. Andres. 1991. *Eustenopus villosus* (Coleoptera: Curculionidae) for biological control of yellow starthistle (Asteraceae: Cardueae) in North America. Environ. Entomol. 20(4):1189-94.

Fornasari, L., and R. Sobhain. 1993. Life history of *Eustenopus villosus* (Coleoptera: Curculionidae), a promising biological control agent for yellow starthistle. Environ. Entomol. 22: 684-692.

Sobhian, R. and H. Zwölfer. 1985. Phytophagous insect species associated with flowerheads of yellow starthistle (*Centaurea solstitialis* L.). Z.Angew. Entomol. 99:301-21.

Turner, C.E. and L. Fornasari. 1992. Biological control of yellow starthistle (*Centaurea solstitialis* L.) in North America. In: Proceedings of the Eighth International Symposium on Biological Control of Weeds. Delfosse, E.S. and R.R. Scott, eds. 2-7 February 1992, Lincoln University, Canterbury, New Zealand.

Larinus curitus

Clement, S. L. 1990. Insect natural enemies of yellow starthistle in southern Europe and the selection of candidate biological control agents. Environ. Entomol. 19:1882-88.

Fornasari, L. and C.E. Turner. 1992. Host specificity of the Palearctic weevil *Larinus curitus* Hochut (Coleoptera: Curculionidae), a natural enemy of *Centaurea Solstitialis* L. (Asteraceae: Cardueae). In: Proceedings of the Eighth International Symposium

on Biological Control of Weeds. 2-7 February 1992. Lincoln University, Canterbury, New Zealand, 385-391.

Sobhian, R. and L. Fornasari. 1993. Biology of *Larinus curitus* Hochhut (Coleoptera: Curculionidae), a European weevil for biological control of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae), in the United States. Biol. Control 4: 328-335.

Urophora sirunaseva

Clement, S. L. 1990. Insect natural enemies of yellow starthistle in southern Europe and the selection of candidate biological control agents. Environ. Entomol. 19:1882-88.

Sobhian, R. 1993. Life history and host specificity of *Urophora sirunaseva* (Hering) (Dipt., Tephritidae), an agent for biological control of yellow starthistle, with remarks on the host plant. J. Appl. Entomol. 116: 381-390.

Turner, C. E. 1994. Host specificity and oviposition of *Urophora sirunaseva* (Hering) (Diptera: Tephritidae), a natural enemy of yellow starthistle, pp. 31-36. Proceedings of the Entomological Society of Washington. 96(1): 31-36.

Turner, C. E., R. Sobhian, D. B. Joley, E. M. Coombs and G. L. Piper. 1994. Establishment of *Urophora sirunaseva* (Hering) (Diptera: Tephritidae) for biological control of yellow starthistle in the western United States. Pan. Pac. Entomol. 70: 206-211.

White, I.M. and S.L. Clement. 1987. Systematic notes on *Urophora* (Diptera, Tephritidae) species associated with *Centaurea solstitialis* (Asteraceae, Cardueae) and other palaearctic weeds adventive in North America, pp. 571-580. In: Proceedings of the Entomological Society of Washington.

White, I.M. and S. L. Clement. 1989. A revision of the western Palaearctic species of *Urophora Robineau-Desvoidy* (Diptera: Tephritidae). Syst. Entomol. 14:327-374.

Zwölfer, H. 1969. *Urophora sirunaseva* (HG)(Dipt.: Trypetidae), a potential insect for the biological control of *Centaurea solstitialis* L. in California. Tech. Bull. CIBC 11:105-154.

Vegetation monitoring

BLM: National Applied Resource Sciences Center, Ed. 1996. Sampling vegetation attributes. Cooperative Extension Service, USDA Forest Service, Natural Resource Conservation Service, Grazing Land Technology Institute and USDI Bureau of Land Management. BLM/RS/ST-96/002+1730.

Campbell, C.L. and J.P. McCaffrey. 1991. Population trends, seasonal phenology, and impact of *Chrysolina quadrigemina*, *C. hyperici* (Coleoptera: Chrysomelidae), and *Agrilus hyperici* (Coleoptera: Buprestidae) associated with *Hypericum perforatum* in northern Idaho. Environ. Ent. 20:vol. 1:303-315.

Hironaka, M. 1985. Frequency approaches to monitor rangeland vegetation. Proceedings 38th Society Range Management Conference at Salt Lake City, Utah, February 11-15: 84-86.

Glossary

achene	Seed.
alternate	Leaves that are arranged singly up the stem; not opposite each other.
annual	Plant that germinates, flowers, seeds, and dies during one growing season.
basal	At the base of a plant or plant part.
bolting	Plant stage at which the stalk shoots upward.
bracts	Leaf-like structure at the base of flowers or leaves.
cairn	Mound of stones set up as a marker.
capitulum	Seed head.
coordinates	Any of a set of numbers used to specify a point on a line.
cotyledons	The first leaf-like structures that appear after germination; seed leaves.
density	Number of individuals or units per unit area.
desiccate	To dry up or dehydrate.
diapause	Period of dormancy.
dissemination	Dispersal or spreading of seeds.
duff	Partly decayed organic matter on the forest floor.
elytra	Protective front wing.
emergence	Act of adult insect leaving the insect case.
exoskeleton	The hard, supporting structure on the outside of the body of an insect.
floret	One of the small flowers forming the head of a composite plant.
frass	Plant fragments, usually mixed with excrement.

fusiform	Tapering toward each end.
gall	Swelling of plant tissue.
grub	A soft thick worm-like larva of an insect.
head	A group of flowers borne tightly together
inflorescence	Arrangement of flowers on an axis.
instar	The stage of an insect between successive molts.
involucral	Flower cluster or fruit.
larva (pl. larvae)	Immature insect stage between the egg and pupa.
lobe	A cut into a leaf from the edge toward the center.
mandible	First pair of jaws in insects.
membranous	A thin soft pliable layer.
molt	Process of shedding the exoskeleton.
mottling	Surface having colored spots or blotches.
organdy	A fine transparent piece of muslin or cloth.
overwinter	To survive the winter.
oviposit	To lay or deposit eggs.
ovule	An egg in the early stage of growth.
pappus	A tuft of hairs or bristles often found at the ends of fruits or seeds.
peripheral	Outside border.
phenology	Relationship between climate and periodic biological phenomenon.
plume	A hair-like or feather-like structure, often on a seed.
pubescence	The hairs on a leaf, stem, or flower.
pupa (pl. pupae) (v. pupate)	Non-feeding, inactive stage between larvae and adult in insects.

quadrat	A specific geometric area used in sampling vegetation (e.g., 1 square meter).
qualitative	Measurement of descriptive elements (e.g., age class, distribution)
quantitative	Measurement of quantity or amount.
receptacle	End of the flower stalk with the reproductive organs.
rosette	A circular, normally basal, clump of leaves.
senescence	Last stage of life cycle.
snout	Anterior prolongation of the head of a weevil
spine	A stiff pointed plant part.
synchrony	Happening at the same time.
thorax	Body region behind the head
transect	A narrow area, of varying width, used to measure quantitative samples.
variable	A quantity that has any one of a set of values.
x-axis	Vertical axis or line in a coordinate system.
y-axis	Horizontal axis or line in a coordinate system.

Appendices

Appendix A: University of Idaho Agent Release Form



Agent Release Form Bio Control of Yellow Starthistle

RELEASE SITE INFORMATION

Site name _____

State _____

County _____

Nearest town _____

Road/mile marker _____

Landowner BLM USFS Private: _____
 NPS State Other: _____

Legal Description _____

Township _____

Range _____

Sec _____

GPS _____

Latitude (Deg) _____

(Min) _____

(Sec) _____

Longitude (Deg) _____

(Min) _____

(Sec) _____

RELEASE SITE DATA

Slope none slight moderate steep Aspect south east west north

Soil sandy loam silt gravel clay Elevation _____

Topographic position valley foothill mountain plain river crest

Plant cover (Estimate %) target weed _____ forbs (not including target) _____ grasses _____
 shrubs _____ trees _____ litter _____ bare ground _____

Disturbance grazing logging road mining cultivation construction fire flood

Target weed _____ Ave. height _____ Ft. Est. weed density _____ /sq.m

Distribution isolated scattered patchy continuous Size of infestation (acres) _____

AGENT RELEASE INFORMATION

Agent	<input type="checkbox"/> <i>Bangasternus orientalis</i>	<input type="checkbox"/> <i>Eustenopus villosus</i>	<input type="checkbox"/> <i>Larinus curtus</i>	Release date
-------	---	---	--	--------------

Released	<input type="checkbox"/> <i>Chaetorellia australis</i>	<input type="checkbox"/> <i>Urophora sirunaseva</i>	Time of release
----------	--	---	-----------------

Released by	Agency
Address	Phone Fax E-mail

Source of agents	Collection date	Collection location T _____ R _____ S _____
	Collected by	Lat Long

No. released	Weather conditions
Agent stage at release	<input type="checkbox"/> egg <input type="checkbox"/> larvae <input type="checkbox"/> pupa <input type="checkbox"/> adult <input type="checkbox"/> vegetative material
Weed stage at release	<input type="checkbox"/> seedling <input type="checkbox"/> rosette <input type="checkbox"/> bolting <input type="checkbox"/> BU1 <input type="checkbox"/> BU2 <input type="checkbox"/> BU3 <input type="checkbox"/> BU4 <input type="checkbox"/> flowering <input type="checkbox"/> seed formation <input type="checkbox"/> mature <input type="checkbox"/> seed dissemination <input type="checkbox"/> senescence

Biology and Biological Control Agents of Yellow Starthistle

Other agents present (list)

Directions to release site

Please draw a map to the release site indicating the release site with an 'X'; label landmarks:

Comments

★★IMPORTANT ★★ Please retain a small sample of the agent released for a voucher specimen and mail to the University of Idaho address below. ★★IMPORTANT ★★

**PLEASE
MAIL THIS
FORM
WITHIN TWO
WEEKS OF
RELEASE
TO:**

**Joe McCaffrey
University of Idaho
Department of Plant, Soil and Entomological Sciences
Moscow, ID 83844-2339**

**Phone: 208-885-7548
FAX: 208-885-7760
E-Mail: jmccaffrey@uidaho.edu
<http://www.ets.uidaho.edu/pest>**

Appendix B: Monitoring Plan Checklist Form

Monitoring Plan Checklist Form

The following is a list of questions to be answered and documented prior to collecting monitoring data. Use the checklist as an outline for a monitoring plan.

What is the management objective? _____

What is the monitoring objective? _____

What is going to be measured? _____

What is the appropriate level of monitoring intensity? _____

What is the population to be monitored? _____

Where will the monitoring take place? _____

When will the monitoring be conducted (month/year)? _____

How often will monitoring be conducted? _____

Who will conduct the monitoring? _____

How much will the monitoring cost (include summarizing and analyzing the data)? _____

How are the field data sheets to be designed? _____

What equipment and supplies are needed? _____

What training is necessary? _____

Appendix C: Biocontrol Monitoring Report

BIOCONTROL MONITORING REPORT

Release Site Location:

State: _____ County: _____

Site name: _____

USGS coordinates: TR _____ Sect _____ Qtr-sect _____
Latitude: _____ Longitude: _____ (GPS) derived: Y _____ N _____

NOXIOUS WEED

Species of noxious weed: _____

BIOCONTROL AGENT RELEASED

Species released: _____ Date of original release: _____

MONITORING INFORMATION

Sampling Date: _____ Sampling Time: _____

Weather conditions: Clear _____ Partly cloudy _____ Overcast _____ Rain _____ Other (specify: _____)

Air temperature (°F): <60 _____ 60-70 _____ 70-80 _____ 80-90 _____ >90 _____ (Actual temp., if measured: _____)

Wind: Calm _____ Light _____ Moderate _____ Strong _____

Biocontrol Sampling

Visual observation of insect before sampling? Y _____ N _____

Number of net sweeps: _____ Total number of agents swept (sum from reverse): _____

Visual observation of insect/5 minutes: 0 _____ <2 _____ 2-5 _____ 6-10 _____ >10 _____

Total number of seed heads sampled: _____ Number of seed heads infested: _____

Total number of roots sampled: _____ Number of roots infested: _____

Estimate of population level: established _____ marginally collectable _____ collectable _____

Vegetation Sampling

Photos taken? Y _____ N _____

Dominant plant: _____ Target Weed: _____

Percent cover of total vegetation: Tree _____ Shrub _____ Forb _____ Grasses _____ Litter _____ Bare Ground _____

Type of sample: Daubenmire frame _____ Other (Specify): _____

Number of samples taken: _____ (minimum of 12 Daubenmire frames)

Average number of stems of target weed per sample: _____ (average from reverse side)

Average percent cover of target weed per sample: _____ Average height _____

Radial distance of "Bomb-Blast" effect (feet): N _____ S _____ E _____ W _____

Observer: _____

Affiliation: _____

Phone: _____

SAMPLING INSTRUCTIONS

* VEGETATION SAMPLING: Establish 4 transects, 1 in each cardinal direction, intersecting at the release point. Place a Daubenmire frame to the right side of each transect line at a point 5, 10 and 15 feet from the release site. Count the number of stem within the frame. Record the average height and the percent cover occupied by the target weed. Record this information below.

* SWEEPING: First, look over the release area to see if biocontrol insects are evident. Next, sweep 3 sampling points along four lines in N, S, E, and W direction from the release point (12 points total). For each line, begin 5 feet from the release point. Using a 15-inch diameter net, make 4 sweeps in front of you. Carefully examine the net and count the biocontrol agents present, then empty the net behind you to release counted insects. Move 5 ft out and repeat above steps. Continue until 3 points have been sampled, then repeat over the remaining cardinal directions. A diagram of the sampling procedure and a chart on which to record insect counts is provided on this form.

* VISUAL: Sit quietly for 5 minutes in the infested area near the release point and look for the insects. If you see none, then carefully and slowly move the plants aside to look under the leaves and on the stems.

* SEEDHEAD COLLECTIONS: Collect seed heads by manually snapping the seed heads off the plants. Randomly collect 200 seed heads. Place the collected seed heads in a paper bag and when convenient count the number of infested seed heads and the total number of seed heads sampled.

* ROOT COLLECTIONS: Dig roots from at least 20 randomly collected plants. Place the plants in a cooler. When convenient search the roots or split the roots and look for the biocontrol agent. Count the number of infested plants. If 25% or more of the plants are infested then the population is probably collectable.

MONITORING LAYOUT

VEGETATION MONITORING (Sample should be taken on the right side of transect with back to release point facing in the appropriate cardinal direction)

		Direction			
Distance from release point		N	S	E	W
	Number of stems of weed				
5'	Average Height of weed				
	Percent Cover of weed				
	Number of stems of weed				
10'	Average Height of weed				
	Percent Cover of weed				
	Number of stems of weed				
15'	Average Height of weed				
	Percent cover of weed				

INSECT COUNTS (Sample should be taken on the left side of the transect with back to release point facing in the appropriate cardinal direction.)

		Direction			
Distance from release point		N	S	E	W
	5'				
	10'				
	15'				

Appendix D: Qualitative Monitoring Form

Qualitative Monitoring Form

Date: _____ Time _____ a.m./p.m. Examiners: _____
Location: _____ Site #: _____
Insect released at site _____ Year of release: _____

Cover Class by Life Forms						
	0%	1-5%	6-20%	21-45%	46-70%	71-100%
Y. Starthistle						
Annual Grasses						
Perennial Grasses						
Forbs						
Shrubs						
Trees						

Dominant Plants on Site
Other Noxious Weeds:

Yellow starthistle density class (✓ check one)		
(Flowering plants/meter sq.)	YST distribution	
0	Isolated	
1-25	Scattered	
26-50	Scattered-Patchy	
50-75	Patchy	
>75	Continuous	

Phenology class at time of monitoring	
YST stage	Estimated Percent
Rosette	
Bolting	
BU-1	
BU-2	
BU-3	
BU-4	
Flower	
Senescence	

Comments/Observations _____

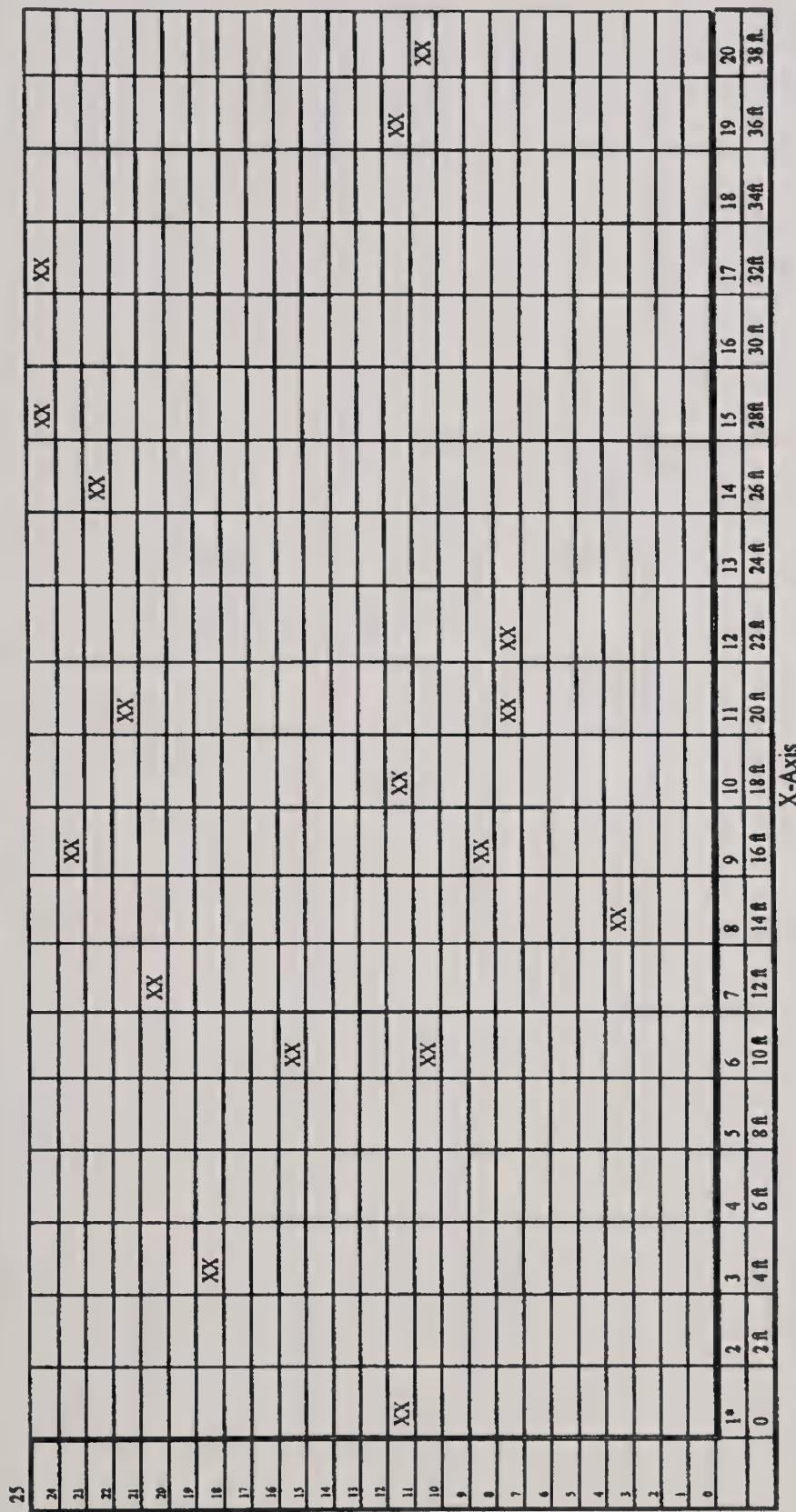
Appendix E: Quadrat Density/Cover Data Form

Quadrat Density/Cover Data Form

Appendix F: Example of Macroplot Design for Sampling Density

Example of Macropot Design for Sampling Density

Designed for a 1 X 2 ft quadrat 25 X 40 ft. Macropot



* quadrat coordinate number
XX = quadrat to be sampled.

Appendix G: Oregon Biological Control Monitoring Form

Biological Control Monitoring Form Instructions

Oregon Department of Agriculture Biological Control

Date	Enter date as MM/DD/YY (e.g., June 4, 1997, is 06/04/97).
Weed	Enter weed code, first two letters of genus and species (e.g., yellow starthistle, <i>Centaurea solstitialis</i> , is CESO).
Agent	Enter the agent code, first two letters of genus and species (e.g., <i>Urophora sirunaseva</i> is URSI).
County	Enter the first four letters of the county (e.g., Latah County is LATA).
Location	Enter the Latitude and Longitude in Decimal Degrees from your GPS unit (top line) and Township Range and Section (bottom line). If you do not have a GPS unit, you will have to convert T&R to LATLONG with the Cahis program. Make sure that your GPS is set for decimal degrees. Do not exceed four places beyond the decimal.
Manager	Who manages the land? This is important for contract reporting. Examples: BLM, FS, ODOT, BPA, USFWS, PRI (Private), CO (County), CITY etc.
District	Who has local jurisdiction over the land? This would be National Forest, BLM District, ODOT Highway, County Road, BPA line, etc.
Stage	What stage of a biocontrol agent did you find? A= adult, L= larva, P= pupa, E= egg. You can list multiples, or dominant life stage observed.
Abundance	How abundant is the biocontrol agent? Express how many you can find, catch, etc., in one minute, no matter what method. If there are a lot, then estimate the number as best you can.
Infested %	What percentage of the whole plants that you checked had been attacked by the biocontrol agent? You can check whatever number of plants you feel is adequate to get a general idea of the infestation rate. Try to check at least 10+ plants at a site. Once you find an infested seed head, the plant is attacked.
Density	What is your best estimate of the weed density per square meter for the major part of the site?
Area	What is the approximate size of the infestation in acres?

Oregon Department of Agriculture Biological Control - Monitoring Form

Name: _____

Acknowledgments for Photos and Drawings

Cover	Cindy Roché, University of Idaho
Fig. 1	Cindy Roché, University of Idaho
Fig. 2	John Connett, University of Idaho
Fig. 3	Cindy Roché, University of Idaho
Fig. 4	W.M.C., University of Idaho, CIS # 445
Fig. 5	Weed Science, University of Idaho
Fig. 6	Weed Science, University of Idaho
Figs. 7a-j	Cindy Roché, University of Idaho
Fig. 7-k	University of Idaho
Fig. 7-l	Cindy Roché, University of Idaho
Fig. 8	University of Idaho
Fig. 9	University of Idaho
Fig. 10	Eric Coombs, Oregon Department of Agriculture
Fig. 11	Eric Coombs, Oregon Department of Agriculture
Fig. 12	John Connett, University of Idaho
Fig. 13	John Connett, University of Idaho
Fig. 14	University of Idaho
Fig. 15	Gary Piper, Washington State University
Fig. 16	John Connett, University of Idaho
Fig. 17	J. B. Johnson, University of Idaho
Fig. 18	J. B. Johnson, University of Idaho
Fig. 19	John Connett, University of Idaho
Fig. 20	John Connett, University of Idaho
Fig. 21	John Connett, University of Idaho
Fig. 22	Linda Wilson, University of Idaho
Fig. 23	Leonard Lake, US Forest Service
Fig. 24	University of Idaho
Fig. 25	Gary Piper, Washington State University
Fig. 26	Cynthia Jette, University of Idaho
Fig. 27	Dennis Schotzke, University of Idaho
Fig. 28	Eric Coombs, Oregon Department of Agriculture
Fig. 29	Eric Coombs, Oregon Department of Agriculture
Fig. 30	Eric Coombs, Oregon Department of Agriculture
Fig. 31	Cynthia Jette, University of Idaho
Fig. 32	University of Idaho
Fig. 33	Cynthia Jette, University of Idaho
Table 1	California Department of Food and Agriculture, Sacramento, CA
Table 3	Cynthia Jette, University of Idaho



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